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The 1983 Portland Giardia Conference was held on November 14, 15 and 16, 1983 in Portland, Oregon. National experts on giardia and giardiasis were invited, as well as state and local water officials and purveyors. The people attending had two roles: to hear presentations and to work together as contributing experts in small groups on giardia-related topics. The topics were as follows:

Public information
Testing for giardia in water
Financing for water system improvements
Water system evaluation/risk evaluation
Control measures for giardia in water systems
Surveillance for waterborne giardiasis
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EPA 1983 PORTLAND GIARDIA CONFERENCE

NOVEMBER 14, 15 and 16, 1983

REPORT

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INTRODUCTION AND ACKNOWLEDGEMENTS

The 1983 Portland Giardia Conference was held on November 14, 15 and 16 in Portland, Oregon. National experts on Giardia and giardiasis were invited, as well as state and local water officials and purveyors. The people attending had two roles: to hear presentations and to work together as contributing experts in small groups on Giardia-related topics. The topics were as follows:

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Control Measures for Giardia in Water Systems
Surveillance for Waterborne Giardiasis
Future Regulatory Requirements
Future Research Needs

We couldn't have put on this conference without the valuable help of many people. Specifically, I would like to thank the members of the Conference Steering Committee: Larry Foster, Mark Knudsen, Larry Eisele, John Stoner and Jim Boydston; and the expert speakers Charles Hibler, Dennis Juranek, Larry Foster, Byron Plan, Floyd Frost, Ed Lippy, John Kirner, Rick Karlin, and Jay Vasconcelos. Larry Foster, Byron Plan and Rick Karlin also deserve special thanks for their assistance with the agenda and throughout the conference. Finally, I wish to acknowledge the contribution of Jean Knight, formerly of the EPA's Oregon Operations Office. Her tireless work brought the conference to reality.

The following pages provide summaries of the information presented by the speakers and generated by the work groups. Each is given as a short report, a few with added appendices.

We were very pleased with the discussion and free exchange of ideas that took place in the conference. The group reports and other products of the conference will prove useful in the continuing effort to ensure safe drinking water in Oregon. We present this information in the hope that it will prompt further discussion, research, experimentation and perhaps more answers. Please feel free to contact us if you have comments or questions, and to reproduce this book (or parts of it) and pass it on to others.

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OPENING REMARKS

The conference began with a welcome by John Vlastelicia, Director of the Oregon Operations Office for E.P.A.'s Region 10.

Welcome to Portland. Let me thank you all for coming to our Giardia Workshop. It is an important effort for us, and we appreciate your willingness to help.

We have an ambitious agenda. Based on your expertise, input, consideration of the specific problems, and recommendations, we expect to close this workshop on Wednesday having agreed on enough to help us solidify an approach to limiting waterborne giardiasis in Oregon.

Let me briefly review where we are, and where we're headed. The EPA is responsible for regulation of public water supplies in Oregon. Our approach is primarily regulatory: i.e., to assure compliance with the maximum contaminant levels and reporting requirements, and to resolve violations via regulation or enforcement.

The Oregon State Health Division has emerging a strong, technical-assistance-oriented program to help water suppliers prevent problems. They also have an epidemiological group to address the disease-outbreak aspects of drinking-water contamination. We have county health departments with resources and expertise ranging from insignificant to very good, which gives us many hands to help to provide safe drinking water in Oregon.

But even though there are enough agencies involved, we still have Giardia and giardiasis in Oregon. Inadequately treated surface-water supplies appear to be a major part of the problem. We have nearly 3,000 public-water supplies in Oregon, a third of which serve year-round residents. About 200 of those are surface-water supplies that serve a million and a half people. Less than a hundred provide full treatment (that is, filtered water). And, as we all know, when you don't have filtration on surface-water systems, you often see Giardia in the finished water.

Oregon has had several confirmed waterborne giardiasis outbreaks in recent years, and probably as many not confirmed. While the statistics are not overwhelming, it is the leading waterborne disease in Oregon. For example, in 1982 there were 737 reported cases of giardiasis in Oregon. As of late October, 1983, cases are running 100 more than at the same time last year. We have no information to say how many of these are related to the drinking-water system, but certainly some are. And we don't know which systems are at risk, nor do we know where to expect the next outbreak.

We want to improve our management approach to Giardia control. You've heard of management Theory X and Theory Y? Sometimes I think we use the "Double O" Theory for Giardia management. That is: over a period of weeks, the agencies involved slowly realize a concentration of giardiasis is emerging, and it appears to be occurring within a unfiltered surface-water system. Then a Giardia cyst is isolated in a stool, and then in the water. Under the "Double O" Theory approach, we then exclaim together, "Oh Oh!" This is also referred to as "Case by Case

Management," only it usually happens after the cases occur. We also find varying professional opinions about cause, effect and cure -- and about the degree of "proof" required in the minds of agencies and water-systems operators. (Some of this is simply difference of professional opinion, occasionally even based on the same facts.)

This does not mean that giardiasis problems are not addressed. They are. Our few confirmed *Giardia* outbreaks have been resolved; through cooperation, good communication, and (to a certain extent) crisis management. There have been "boil-water" advisories, increased chlorine requirements, lengthened contact times, alternate-source development, and in some cases, a "wait 'til it passes" approach.

But most of our solutions have been a matter of consensus-building *after* the outbreak. Under Theory O, the degree of success we have in dealing with *Giardia* depends on consensus-building once in the field. While that's possible, it's also difficult. We are convinced that consensus-building *before* an outbreak is the key to improved *Giardia* response.

That's the purpose of this workshop. Despite the fact that a lot about *Giardia* and waterborne giardiasis is still unknown, our principal interest here is to examine what we do know, and to come to some agreement about it. Consensus on various *Giardia*-related issues will help us all when facing day-to-day *Giardia*-related decisions, be they evaluating giardiasis risk, monitoring for its potential or its occurrence, defining outbreaks at early stages, dealing with it in the short and long run, or preventing it via treatment.

Most of what we need to know has a spokesperson in this room. Each of you brings a particular expertise, perspective, and interest to this conference. Among your experiences are the essential elements necessary for consensus about *Giardia* and its management. We need this information to improve our present approach, and to reduce giardiasis occurrence in Oregon and elsewhere.

Since the knowledge is already here, the challenge is to identify the elements of what we know about *Giardia* and to reach consensus. I guess that's why it's called a workshop: everyone works. The seminar is organized to accomplish these objectives through small-group sessions and expert presentations.

Giardia is a vital issue for us in Oregon. (I suspect it is for you too, since beavers don't recognize state boundaries.) We look forward to your input. We also hope you will leave with more than what you bring: that this experience will provide some additional worthwhile information for you to apply to your own programs.

Thank you again for your help. I wish you a productive, enjoyable workshop.

GIARDIA LAMBLIA AND GIARDIASIS

Drs. Chuck Hibler and Larry Foster gave presentations on two aspects of Giardia. Following are summaries of their presentations.

Giardia, the Organism

(Charles Hiber, D.V.M., Ph.D. Wild Animal Disease Center, Colorado State University)

Species of Giardia are protozoan parasites found commonly in many animals, and those reported and described from each animal have been assigned appropriate names (e.g., Giardia lamblia from man, G. canis from the dog, G. cati from the cat, and G. bovis from cattle, to name a few). However, Filice (1952) undertook a detailed study of the species reported from various animals and concluded that only two species exist. Giardia muris with rounded median bodies occurs in mice, rats and hamsters, while G. Duodenalis (the type species from the rabbit) with claw-shaped median bodies occurs in many animals, including various species of rodents, ruminants, carnivores, primates and man. Filice also discovered that only a limited number of acceptable cross-transmission studies had been performed. Consequently, we are aware that many species of animals are infected, but we do not know the host range or host specificity of the organisms.

Giardia leaves the host in a cyst form, a more-or-less resistant stage. This cyst is oval, and measures 7-8 x 10-12 microns. Within the cyst, the infective organism contains four nuclei, four median rods referred to as axostyles, and two median bodies that are claw-shaped in the G. duodenalis type, rounded in the G. muris type. This cyst is immediately infective upon discharge from an animal. When ingested by a susceptible host, the cyst is activated in the stomach and the organism released in the small intestine. This infective form divides immediately by binary fission to form two vegetative forms or trophozoites. All multiplication occurs by binary fission. The trophozoite is pear-shaped, measuring approximately 15 x 20 microns. It possesses four pairs of flagella and a ventral sucking disc, giving the appearance of a partially scooped-out pear. Internally, the trophozoite possesses two nuclei, two axostyles and two median bodies.

Animal surveys have shown that a number of wild and domestic animal species are infected with Giardia. Our data, together with information found in other surveys, indicates that up to 51% of the beaver and 80-90% of the muskrat are shedding cysts in the late summer and fall months. We have found cysts in cattle (about 10%), domestic sheep (about 4%), captive moose calves (5 of 6) and captive mule deer fawns (about 15 fawns). We have also found cysts in coyotes, feral dogs and cats and in human stool specimens from campsites. Although we have not found Giardia in the various small wild rodents that abound around streams, other investigators have reported a high percentage infected. We did not find Giardia in any of about 150 horses

Giardia, the Organism (Continued...)

drinking water from infected streams, but it has been found in one horse in Lolo National Forest, Montana (verified by CPH). We have not found Giardia in free-ranging elk, mule deer, bighorn sheep and pronghorn antelope, or foxes, badgers, skunks, etc.

We examine several thousand dog and cat stools annually at the Veterinary Diagnostic Laboratory. We find that approximately 20-25% of all dogs and 2% of all cats (routine stool examination) are clinically infected with Giardia. If the animal (dog) presents with diarrhea, and is less than 6 months of age, 85% of the time the cause is Giardia. Postmortem examination of dogs through the necropsy laboratory has shown that about 90% of the dogs are carrying Giardia but are not passing cysts, at least in numbers sufficient for routine recovery.

We do not know the role of the wild and domestic ruminants, carnivores, and most rodents, as reservoirs for Giardia and as potential causes of waterborne giardiasis among backpackers, hikers and in municipalities. The beaver and (possibly) the muskrat, because of the habits, habitat, and the high percentage infected are the most logical reservoirs for a continuous source of the large numbers of cysts necessary for an outbreak. Regrettably, no cross-transmission experiments have been performed to determine if muskrat-source Giardia are infective for man.

Most of the cross-transmission studies on host specificity of Giardia were performed at Colorado State University. In summary, we have shown that the Giardia species found in man is infective for dogs, cats, beaver, pronghorn antelope, bighorn sheep and various laboratory rodents. We have also shown that Giardia from dogs, beaver and mule deer is infective for man. We have been unable to infect cattle, elk, and domestic sheep with Giardia from humans, but we feel that these failures were logistical in nature (these ruminants were not thirsty when orally exposed). We had variable success with many of the above animals, some of which possibly can be attributed to the previous immunity, concurrent infection with other flagellates (Trichomonas muris), uninfective cysts, age, diet, etc. Moreover, we often had limited numbers of known negative animals available for trials. Much more host specificity research needs to be done, especially with the small rodents that have an aquatic habitat, and especially with muskrat because they are so ubiquitously infected and so common on watersheds.

Q. "Which of these animal species do you think have been inadequately sampled to really come to any conclusions - wild animal samples that is?"

A. "I think that many animals have been inadequately sampled in the West."

Giardia, the Organism (Continued...)

Q. "What kind of samples are you talking about? Are you talking about just going out and picking up droppings?"

A. "No. We do a lot of post mortems, as I said: we do about 500 post mortems a year, on many of these animals. We get in an animal and I have to be there anyway, so some of my students are always on hand to do a direct smear on the small intestine. I might add that we have found it recently in six of seven black-crowned night-herons passing through Ft. Collins. They had more Giardia than I have seen in anything other than moose calves. The moose calves we saw were captive at Syville, Wyoming. They were on a stream, on which there were infected beaver. Five of the six moose calves had tremendous numbers of cysts in the feces. They were stacked five deep on a cover slip. Most of the calves were showing very severe clinical giardiasis. Probably they were compromised because they were raised in captivity and their thymus gland was not as functional as it should have been."

Q. "Do you know if anyone has tried to infect the muskrat with human Giardia?"

A. "We would like to do so. We have had a study underway. Thus far, we have not found a muskrat we are confident has never been infected, or is not infected and is in the carrier state. So far as I know, no one has tried it. We are reluctant right now because every muskrat we have examined is infected, 80% to 90% infection. Of those that are in the negative category, post mortem analysis was such that we couldn't say for sure. We did call them negative, since we couldn't find the organism. I feel, to follow Dr. Meyer's question, that the muskrat may be far more important than we have considered previously."

Q. "Have you done any work, or is there any information available on rodents as carriers, and has the field mice population been evaluated?"

A. "Of those we looked at in Colorado, and we have looked at a tremendous number of these little rodents, we have yet to find a single Giardia cyst. However, the material I have from Lolo National Forest (I think about 20 samples) three were infected, and two I could recognize as the Giardia muris-type organism. The muskrat samples that we get from Lolo do have the Giardia lamblia - type organism."

Q. "You have oppossums in Colorado?"

A. "No."

Q. "You mentioned that there was kind of a seasonal trend in terms of the infection rate. How many years did you look at those figures?"

A. "Three years."

Giardia, the Organism (Continued...)

Q. "Regarding beavers, this trend of stool/pond activity sounds plausible with all tourists. I was wondering if you looked at the natural history of the organism in the beaver itself: whether the beaver that gets infected this summer is going to have it next summer?"

A. "No, we haven't. It's difficult to trap and tag a beaver with all the beaver-trapping activity going on in Colorado."

Giardiasis, the Illness

(Larry Foster, M.D., Oregon State
Health Division, Portland, Oregon)

The charts Larry refers to are in the body of his outline, presented in Appendix B.

We may see a range of clinical pictures with giardiasis. At the top of the list is asymptomatic infection: I think this is important for all of us to consider because of the potential public-health importance of the asymptomatic infection, particularly in the day-care setting. The literature has not described satisfactorily the natural history of asymptomatic infection in humans. In earlier experiments, a group of 14 people were followed after experimental infection. All of them cleared their parasites within 41 days. Some of them had a symptomatic illness, and some didn't. If we were to judge from these limited observations, it would seem that the asymptomatic infected state persists for a relatively short period of time. Yet anecdotal evidence we collect monthly from day-care centers here in Oregon and elsewhere certainly suggests that the asymptomatic infected state persists for a very long time. I think this is an area in which we need a great deal of study, particularly to help us judge what to do to prevent giardiasis transmission in day-care-center settings.

Next, after asymptomatic infection, is acute clinical illness: the textbook description of giardiasis. Certainly some patients do have the classical presentation, which is: sudden onset of explosive diarrhea which is foul smelling, very little blood or mucous in the stool, a lot of bloating, a lot of gas, and a lot of cramping. Patients with just diarrhea could mimic a diarrheal illness caused by one of many other organisms -- bacteria, parasites, viruses.

The duration of the illness also needs further study. For a case definition in Oregon epidemiological studies, we have recognized the classic symptoms of explosive diarrhea, bloating and cramping. Clearly, we have missed some cases that way because even the literature has reported confirmed cases in people who were ill only three or four days. Long duration of symptoms at least does help us to distinguish giardiasis cases from some other diarrheal illness.

Giardiasis, the Illness (Continued...)

Another interesting area in the study of giardiasis is that of the chronic occurrence of symptoms in the person infected with Giardia. The symptoms are variable in these people, but usually they have intermittent diarrhea and the bloating and cramping. Often, the illness is serious enough to lead to weight loss over a period of months. You hear about people who say, "I've been having this diarrhea off and on for the last seven or eight months, and I've lost 30 pounds." Additionally, a chronic syndrome of intermittent diarrhea has been reported in as many as 10% of people who have been treated for giardiasis and who have apparently cleared the parasite. Some folks have called it the "post-giardiasis syndrome." I'm not sure we fully understand the mechanism of this.

The diagnosis of giardiasis is a very special problem to us that have to deal with epidemic investigations and clinical situations day-to-day, because it is neither easy to do nor cheap. The most frequently used method, of course, is the microscopic examination of the stool, usually using a formalin-preserved stool. This enables us only to identify the cysts. It isn't a 100% method: one study, for example, has said that a single stool specimen, carefully examined, will identify only 76% of people who have giardiasis. A second specimen increased the percentage to 90%, and a third up to around 97%. Other research backed up these figures. So if we are really going after the diagnosis of giardiasis in an individual, we should use three stool specimens. We routinely recommend this, with each specimen to be collected at least 24 hours apart. In the private laboratory here in Oregon, a single stool examination may run $30 to $40, so we are lucky to get even one sample.

Q. "You said collect one stool specimen each 24 hours. Doesn't some of the literature say to leave one to three days between specimens?"

A. "Well, the 24-hour timing is what we consider a balance. That covers 72 hours. We recognize the problem of intermittent shedding, although we don't yet fully understand what to expect in terms of shedding in various stages of clinical illness. We definitely think it is important that all the stools not be taken in one day because of the intermittent shedding. Of course, when we have a person with acute, explosive diarrhea and who is miserable, we want to make a diagnosis as fast as we can. The 24-hour spacing is therefore the compromise we have come to. I'd be eager to hear what folks from the other states do."

PVA or polyvinyl alcohol is the preservative we use for samples from patients who are in the acute phase of illness and we can't find cysts. As I mentioned, formalin won't preserve the trophozoite, but PVA will. It enables us to identify the person excreting mostly or exclusively the trophozoite. A lot of textbooks talk about warm, fresh stools to identify the trophozoite: I think this is a figment of the authors' imaginations. Except for hospitalized patients, have you ever tried to get to the laboratory a fresh, warm stool from a patient who is at home twenty miles away? Of course, in the ideal setting of a hospitalized patient, or the patient who has a bout of diarrhea while at the office, it is possible.

Giardiasis, the Illness (Continued...)

Another promising test that a lot of doctors use now is the string test. The person swallows a gelatin capsule attached to a string. The string is left in for a period of time, then withdrawn. The mucous and material adhering to the string can then be examined for the presence of the organism.

Aspiration of duodenal contents or biopsy aren't done much anymore because stool examination and/or the string test are much less invasive and less expensive.

In respect to the pitfalls of diagnosis, we have already talked about the intermittent shedding problem. I think we should also consider causes of falsely negative stool examination tests for giardiasis. Many things used in the management of diarrheal patients interfere with identification of the organism on the microscopic slide. For example, it isn't unusual for a giardiasis patient to show up having been worked up with a barium enema before the doctor even thought of doing a stool examination for Giardia. The barium can interfere with seeing the organism. Some antibiotics, antacids, anti-diarrheal medications, laxatives, and enema preparations can interfere with the test. Because of this, one should consider the possibility of parasitic infection early in the patient's workup. If you get a negative test on a patient who appears to have giardiasis, be sure to consider that other factors might cause a false negative on a stool examination.

The small table listed under "Treatment" in (Appendix B) is from the Medical Letter. These are the standard treatments used by most physicians in Oregon. I would be interested to hear whether other states follow along with the Medical Letter. I just want to make a few brief comments about each drug.

Quinacrine is probably the most effective of the drugs. Effectiveness rates have been quoted to be around 95% among adults. It's important to note a recent study with children, however: those under age 5 had only a 64% effectiveness rate for eradicating the organism in one course of treatment, compared to 92% in older children. This probably has to do with the bitter taste of the medicine causing non-compliance. It also might have to do with the side-effect of vomiting, which seems to be fairly common in younger kids. In Oregon, we don't really encourage using Quinacrine for younger children, because we don't believe you can get it down and keep it down.

Metronidazole or Flagyl^R is also pretty effective. Effectiveness rates in adults for the standard regimen have been quoted from 89% to 100% in most studies. Single-dose Flagyl^R has been considered. One study using 2.4 grams found only a 50% rate, though. I think it's important to note that Metronidazole is not approved by the FDA for treatment of giardiasis because of concerns about teratogenicity in laboratory animals and mutagenicity in bacteria. So, even though Flagyl^R is probably the more commonly used drug for giardiasis here in Oregon, it isn't approved for that purpose by the FDA.

Giardiasis, the Illness (Continued...)

Furazolidone is the third drug that we have available. This is what we generally recommend for children because it is available in a syrup as well as a tablet. Kids tolerate it better for its taste. You'll note the Medical Letter recommendation describes a seven-day regimen. A study published earlier this year compared a 5-day regimen of furazolidone or Furoxone^R to a 10-day regimen. The effectiveness of the 5-day regimen was only 20%, and that of the 10-day regimen was 92%. We strongly advocate the longer regimen because we've had some experiences with treatment failures in pre-school children.

Another drug that is occasionally mentioned is paromomycin, sometimes suggested for treatment of severely symptomatic giardiasis in pregnancies, as it is minimally absorbed from the intestinal tract. I don't believe any extensive clinical trials have been done with it.

We need to consider reinfection versus compliance with the recommended treatment when we judge whether people are truly treatment failures, and when deciding how to further manage a patient who continues to be symptomatic or continues to excrete Giardia after a course of treatment.

Next, I wish to review the basic modes of transmission for giardiasis. The first, and in my mind, foremost is day-care transmission. I think the better way to say that is "transmission among pre-school children," particularly those that are still in diapers. The Washington survey found over 7% of children ages 1 to 3 to be excreting Giardia lamblia. In South Carolina, a study of rural white first-graders found that among those who had been attending day care, 18.8% were excreting organisms. Only 2.8% of those who had not been in day care were excreting the organism. We are finding here in Oregon that day-care outbreaks pose special problems because of "treatment failures." We're dealing with reinfection, expense, risk of medicating preschool children to control a minimal illness. Lots of judgements have to be made in coming years in making decisions about controlling Giardia in day-care centers.

Household-contact exposure probably does account for transmission, particularly among pre-school children and from them to adults in the family. Sexual contact, particularly among gay men, is becoming a recognized mode of transmission. I won't discuss waterborne transmission, since there will be a session on it later.

I'm aware of only one outbreak of foodborne giardiasis in the literature. It was a situation in which a grandmother changed here grandson's diapers, then took some salmon out of jars and arranged it for her husband to take to work to serve his co-workers. Many of his co-workers developed diarrhea, and were diagnosed to have giardiasis. The grandson was later diagnosed to have probably caused this outbreak. I suspect foodborne giardiasis occurs more often than we recognize it.

Giardia, the Illness (Continued...)

Q. "Do you have any comments on the reliability of the string test?"

A. (Dr. Juranek:) "I can't quote the literature off the top of my head, but they did do a recent comparison of the string test and stool exam on several patients, and the string test was apparently a little more sensitive."

Q. "Do you know what the cost would be for getting the analysis done?"

A. "I think the lab cost would be as much per test as the stool specimen exam. There would be more cost, of course, for the doctor to administer the string test."

Q. "Cryptosporidia is a protozoan that has been suggested to be perhaps even more common than Giardia, and capable of causing human intestinal disease with symptoms resembling Giardia."

A. "That is a really timely comment. Not much has been said about cryptosporidiosis up to this point, and yet right here in Oregon we now have a cluster of cases that look clinically like giardiasis. I think cryptosporidiosis is a diagnosis we need very much to consider and study to see how commonly it occurs here."

Q. "It seems particularly likely that it is occurring because the organism, like Giardia, is prevalent in wild and domestic animals. There is also no reason it can't be waterborne, just like Giardia. So you may have it as a component at some outbreaks where you can't find Giardia in the water. Up to now, nobody's taken a hard look at these unusual organisms."

A. "It does take special techniques to identify Cryptosporidium in the lab, and that's why we may be missing it in some of these Oregon outbreaks."

Q. "What about animal-to-person transmission, such as from a domestic pet?"

A. "I think that's really important. In fact, in most of our giardiasis cases we don't really identify a common source for an outbreak. There must be a fair amount of person-to-person transmission within households or other group settings, and perhaps by contact with infected animals, domestic or otherwise."

Q. (Inaudible)

A. "Oregon Administration rules require the Oregon physician or other health-care provider to report to the local health department that a giardiasis case has occurred. The local health department does an investigation looking for possible sources of exposure that might have public-health importance. Then

Giardia, the Illness (Continued...)

it reports the results of the investigation to the State Health Division. It's only when either the county health department or the State Health Division thinks there may be a cluster of cases with a water system as the source that we do anything about the water system. In that instance, our first step would be to review the water system's records. What do we know about its treatment system? What do we know of its bacteriological history? The second step would be to notify drinking-water program staff, who notify the water purveyor. In cooperation with the EPA, they would get some bacteriological tests, do a sanitary survey, and work with the purveyor. We wouldn't notify the purveyor of an individual case of giardiasis in the community served by his or her water system. Otherwise the purveyor would be getting notified all the time, of cases that have nothing to do with the water system."

To begin the workgroups on testing, Jay Vasconcelos gave a slide presentation about the testing method used in the Region 10 Laboratory. The following pages and Appendix C summarize his talk.

Methods of Testing for Giardia in Water

(George (Jay) Vasconcelos, Regional
Microbiologist, Region 10 Laboratory,
Manchester, Washington)

Background:

Although recent development of an excystation technique by Drs. Bingham, Meyer, Rice and Schaefer could in future lead to developing cultural methods, at this time no reliable methods exist for culturing Giardia cysts from water samples. At present, the only practical method for determining the presence of cysts in water is by direct microscopic examination of sample concentrates.

Microscopic detection in water-sample concentrates isn't an ideal process. Finding and identifying the cysts relies almost entirely on the training, skill, experience and persistence of the examiner. (And it is a skill not widespread among water-supply laboratories.) But despite its limitations, microscopic identification is currently the best method we have.

Years ago, the basic assumption was made that in order to find Giardia cysts in water, some form of sample concentration was necessary. As early as 1956, labs were using membrane filters with a porosity of 0.45 µm. With few exceptions, these attempts were unsuccessful. The center for Disease Control has tried particulate filtration, with diatomaceous earth as the medium. This removed the cysts from the water, but the cysts couldn't be separated from the particles of diatomaceous earth.

With the recent increase in the incidence of waterborne giardiasis, further efforts have been made to improve the detection method. An ideal method would be one that recovers all cysts in a water sample rapidly, cheaply and simply; allows rapid detection, identification and quantification; and provides information on the viability of and/or infectivity potential of cysts detected.

Unfortunately, no such method exists. The methods presently available can be broadly separated into two general stages: primary concentration and processing (see Table 1 on next page), and detection and identification (see Table 2 on next page).

Methods of Testing for Giardia in Water (Continued...)

TABLE 1: PRIMARY CONCENTRATION AND PROCESSING METHODS

METHOD	INVESTIGATOR (S)	RESULTS
1. Membrane Filtration		
Cellulosic (47mm-0.45um)	Chang & Kabler USPHS, 1956	Generally unsucessful
Polycarbinate (293mm-5um)	Pyper, DuFrain & Henry Eng 1982, (unpublished)	Passing 1 gal/min @ 10 PSI. 15-1800 gal total.
2. Particulate Filtration (diatomaceous earth, sand, etc.)	Shaw et al, 1977 Juranek, 1979	Generally good removal but poor eluation
3. Algae (Foerst) Centrifuge	Holman et al, 1983 DHHS, Washington	Good rapid recovery, but limited in field use.
4. Anionic and Cationic Exchange Resins	Brewer, Wright State UN. (unpublished)	Generally unsucessful
5. Epoxy-Fiberglass Balston Tube Filters (10"-8um)	Riggs, CDHS Lab, Berkley, CA (unpublished)	Overall recovery 20-80%
6. Microporous Yarnwoven Depth. Filters (7 & 1um orlon & polyprolylene)	Jakubowski, Erickson, 1979 & 1980, EPA-Cincinnati	Recovery 3-15% Extraction ave. 58%
7. Pellican Cassette System	Millipore Corp. (unpublished)	May be useful for processing filter washings
8. Filterwashing Apparatus	DuWalle, U. of Wash., 1982 (unpublished)	Claims 75% recovery from orlon filters

TABLE 2: DETECTION METHODS

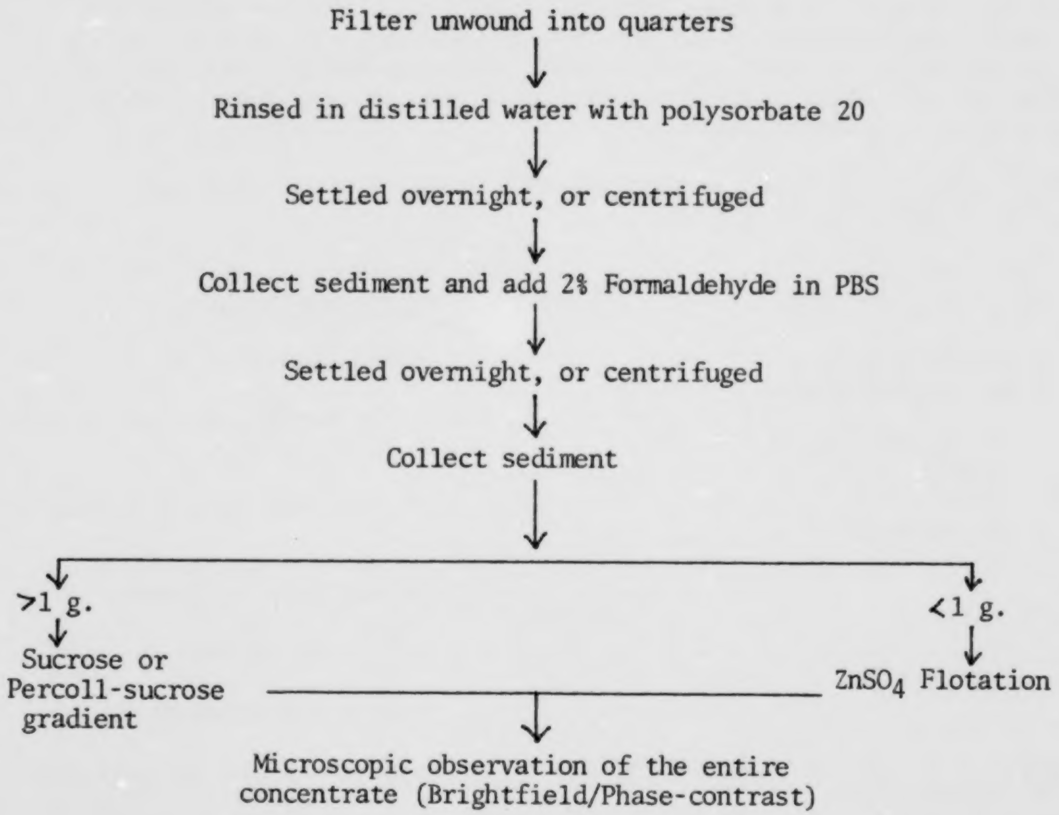
METHOD	INVESTIGATORS(S)	RESULTS
1. Immunofluorescen		
DFA	Riggs, CSDHS Lab, Berkley, CA 1983	Good prep., Cross Rx
IFA	Sauch, EPA-Cincinnati Riggs, CSDS	Still under study
Monoclonal Antibodies	Riggs, CSDHS Sauch, EPA-Cincinnati (unpublished)	Still under study
2. ELISA Method	Hungar, J. Hopkins MD, 1983	Feces samples only
3. Brightfield/Phase Contrast	EPA Consensus method	Ongoing

Methods of Testing for *Giardia* in Water (Continued...)

Copies of Table 1 and Table 2 are also shown in Appendix C, along with further detail about the methods.

EPA Consensus Method:

In September, 1980, the EPA convened a workshop on Giardia methodology in Cincinnati. Its main purpose was to identify the best available methodology, and to agree on a reference method. The five labs in attendance recognized that any proposed method would be based in large part on opinions and personal preferences rather than on hard data, but that agreeing on a consensus method would promote uniformity and provide a basis for future comparisons. Our lab has modified the EPA consensus method slightly for our use. This method is outlined below.


Filter unwound into quarters
Rinsed in distilled water with polysorbate 20
Settled overnight, or centrifuged
Collect sediment and add 2% Formaldehyde in PBS
Settled overnight, or centrifuged
Collect sediment
>1 g.
Sucrose or Percoll-sucrose gradient
<1 g.
ZnSO4 Flotation
Microscopic observation of the entire concentrate (Brightfield/Phase-contrast)

TESTING FOR GIARDIA IN WATER

The topic of Workgroups 2, 3a, and 3b was "Testing for Giardia in Water." The EPA suggested the groups discuss the following questions:

- *What do and don't the tests tell you?*
- *How can testing be used more effectively?*

The EPA also suggested the following possible final products: A list of information that can be gained through Giardia tests. Suggestions for how and when Giardia tests can most effectively be used. A description of needed research on Giardia testing.

Testing for Giardia in Water: Report of Workgroup 2

The group discussed general questions surrounding testing for Giardia, but it was unwilling to go further until the question of whether testing was even useful was addressed. The question defined was, "Is routine testing for Giardia cysts in water appropriate?" The group decided that routine testing was not. Stating the conclusion as a problem gave the following positive and negative aspects:

Problem: Routine testing for presence of Giardia cysts in water is inappropriate.

Positive aspects of routine testing	Negative aspects of routine testing
It's appropriate in some circumstances:	. It's insensitive
. it establishes a baseline of data	. The results are not interpretable
. it establishes a historical record	. It's sometimes unnecessary
	. Question exists about viability of cysts
	. It's expensive
	. It's time-consuming

The group then came up with the following strategies to handle the negative aspects listed above.

- Need to quantify total number to species
- Must improve training of technicians

Testing for *Giardia* in Water: Report of Workgroup 2 (Continued...)

- Should improve sampling to reduce other organics
- Possibly develop specific dye tags/labels for *Giardia*
- Devise trapping techniques with better recovery by the average lab technician

Finally, the group identified three alternatives to routine testing.

- Evaluate risk in the watershed,
- Evaluate biomass instead of testing routinely, and
- Put effective treatment into place.

Testing for Giardia in Water: Report of Workgroup 3a

The group determined the following:

What do tests tell you?

- A positive test means that the water contains Giardia cysts and was fecally contaminated.
- In filtered water, a positive test means there's something wrong with the filtration.
- In untreated water, a positive test means that there may or may not be infections. Adequate treatment is called for.

What don't tests tell you?

- A test doesn't tell the number or concentration of cysts in the water. (You can't quantify results by extrapolating the number of cysts found by a test.)
- If cysts are found, the test doesn't tell whether they are viable (dead or alive).
- Test doesn't tell whether cysts in the water are infectious to humans.
- Test doesn't indicate which species was the carrier.
- A negative test doesn't mean cysts aren't there.

How are tests used most effectively?

- For confirmation in an outbreak.
- To provide guidance for determining the appropriate treatment.
- To establish a data-base showing prevalence of positive Giardia tests.

Research still needed:

- How often should people sample?
- How long should they sample?

Testing for *Giardia* in Water: Report of Workgroup 3a (Continued...)

- Improved methods for detecting *Giardia* cysts in water:
    - a) Better method for isolating cysts
    - b) Better method for detecting cysts during test
- Determine proper holding time and transit conditions.

Testing for Giardia in Water: Report of Workgroup 3b

The group began its presentation by saying, "We didn't come up with any answers, but we did come up with some pretty good questions." The questions follow.

1. Research needs

   a) Need research on removal of cysts from media.

   b) Need a less time-consuming technique for testing water for Giardia; also a less technically difficult method.

   c) Need a better device for monitoring treatment efficacy relative to removing Giardia cysts or other particles/microbes of that size. Could one use an artificial item similar to a Giardia cyst as a monitoring device? Need to determine that technology.

2. Epidemiological observations and questions

   a) Need coordination between EPA's surveillance of water systems and the State's epidemiological surveillance.

   b) Has a protocol been developed to implement epidemiological surveillance throughout the state?

   c) We must realize that Giardia is endemic in the population. What is a baseline of endemic giardiasis?

   d) What causes this organism to become a pathogen? (We know asymptomatic carriers exist.)

   e) Cases vs. carriers: why outbreaks? We determined the following factors could influence the outcome:

      - Compromised hosts - Is the host stressed for some reason, such as a previous illness?

      - Exposure level (dosage of cysts) may vary.

      - The pathogenicity of Giardia subspecies may vary.

   f) Can we immunize against giardiasis?

   g) We need to determine the importance of waterborne giardiasis relative to other methods of transmitting it. (For example, how important is waterborne transmission vs. person-to-person transmission?)

3. Monitoring for Giardia in water systems

   a) Do we need to monitor water systems for Giardia? If so, what type of water systems need monitoring?

   b) It may not be useful to monitor raw or surface water, since all raw or surface water can potentially contain Giardia.

   c) It may be useful to monitor treated water for Giardia to help assess whether a treatment system is working. For systems with limited funds and resources, however, is it reasonable to expect treatment-monitoring for Giardia when the goal is just water quality that meets EPA standards (1 - 5 NTU)?

   d) If you monitor treated water, find cysts, and the cysts are not viable, is there a problem? (Perhaps the treatment kills the cysts.)

   e) If you have full treatment, (i.e., coagulation, flocculation, sedimentation and filtration) and Giardia cysts are recovered, is there a problem with the treatment? Is the problem in the operation of the plant?

   f) Can treatment systems presently in use that are dependent on source adequately remove viable, infective Giardia cysts - or remove them in adequate numbers to prevent disease outbreaks? Can we monitor for this treatment efficacy in some way to provide us with a level of security?

   g) Even if you provide great treatment, it doesn't guarantee that you will prevent outbreaks, because of cross-connections, new connections, et al.

   h) Can we define a treatment regime to control for Giardia in water that addresses such variables as chlorine residual, contact times and turbidity?

4. Miscellaneous observations and questions

   a) Politically, one would have to take action if Giardia cysts are found in finished water.

   b) What is the cost effectiveness of doing something to prevent an outbreak? How safe can we be for the dollars available? What other factors should be taken into account in assessing the cost of an outbreak?

(22

Testing for Giardia in Water: Report of Workgroup 3b (Continued...)

5. Narrowed-down problem

   a) Should we determine which systems could be a problem?

   b) Are adequate monitoring methods available to monitor domestic water treatment or to obtain viable, pathogenic cysts where present? Are these methods cost-effective?

WATERBORNE GIARDIASIS AND ITS EPIDEMIOLOGY

Dennis Juranek, and Byron Plan and Floyd Frost, gave presentations on giardiasis and its epidemiology. Workgroup 8 discussed the question of surveillance for giardiasis.

Waterborne Giardiasis

(Dennis Juranek, D.V.M., M.Sc.,
Center for Disease Control,
Atlanta, Georgia)

Table 1 on the following page summarizes selected epidemiologic characteristics of waterborne outbreaks of giardiasis in the United States. The five outbreaks included in the table were selected as being representative of the majority of outbreaks.

Although outbreaks have many similarities, it must be emphasized that no two outbreaks are exactly the same. Identifying the subtle differences between outbreaks to try to learn the cause often requires a cooperative effort between engineers, water-treatment plant operators, health-department personnel, and epidemiologists. Failure to enlist the assistance of these community, state or Federal resources can impede confirmation and control of a waterborne outbreak. It is important to remember that:

1) Not all Giardia outbreaks are caused by contaminated municipal drinking water.

   (The investigator should first rule out exposure to raw stream water, day-care-center associated infections, homosexual contact, and travel to Giardia-endemic countries.)

2) Not all waterborne outbreaks of diarrhea are due to Giardia.

   (Although when a cause is found, Giardia does head the list of infectious organisms, in most waterborne outbreaks of diarrheal disease reported to the CDC, a causitive agent is never found.)

3) Water filtration is thought to provide an effective barrier against waterborne transmission of Giardia cysts.

   Several outbreaks of waterborne giardiasis have occurred on water systems employing water filtration as part of the treatment process. But it must be emphasized that in every instance, there were either defects in the filters themselves or in the way they were operated, that caused the filter to be ineffective.

Table 1 (Juranek)

Representative Waterborne Outbreaks of Giardiasis (Summary of selected epidemiologic variables)

VARIABLE	1974 Rome, NY Pop. 50,000	1976 Camas, WASH Pop. 6,000	1977 Berlin, NH Pop. 15,000	1980 Red Lodge, MT Pop. 5,000	1982 Reno, NEV Pop. 140,000
Estimated % of population infected	10.6%	4%	5%	15-20%	0.1%
Time of year	Spring	Spring	Spring	Early Summer	Fall
Type of Water	Surface	Surface & Well*	Surface	Surface	Surface & Well*
No. of Surface Sources.	1	2 with one water treatment facility	2 with 2 water treatment facilities	1	1 with 4 water treatment facilities
Origin of surface water	Mountain streams to impoundment reservoir	mountain stream	mountain rivers	mountain	mountain river
Water chlorinated	YES	YES	YES	YES	YES
Amounts adequate for bacterial disinfection	NO	YES - but temporary interruptions	YES - all times	NO	YES - all times
Water filtration	NO	YES	YES	NO	YES - in only 1 of 4 treatment plants
Type of filter		Pressure filter	System 1 - pressure filter System 2 - Gravity sand filter		Gravity sand filter-but not in operation at time of outbreak
Giardia cysts recovered from water	YES	YES	YES	NO	YES
H_2O contamination					
Sewage x-connection	NO	NO	NO	NO	NO
Human	possible	unlikely	possible	possible	possible
animal	possible	likely	possible	possible	likely

*Well water not implicated in outbreak

Giardia and Giardiasis in Washington State

(Byron Plan, M.Sc., Floyd Frost, Ph.D.
Department of Social and Health Services, Olympia, Washington)

Byron presented the history of the problem, the steps taken to begin a concerted investigation into the incidence and extent of waterborne Giardia in Washington, and the results of part of the investigation. Floyd presented the research findings and discussed their implications in terms of public health.

Byron also noted that although the actual project was at an end, the good surveillance process extablished during the research has resulted in their Department still receiving information about human cases, and being involved in epidemiologic giardiasis investigations.

Unfortunately, a transcript of the presentation is not available. But the following abstract, plus two articles co-authored by Floyd and Byron that are in Appendix D, cover the same topic.

Waterborne Giardiasis in Washington State

A project funded by the Health Effects Research Laboratory of the EPA and by the Washington State Department of Social and Health Services examined the epidemiology of giardiasis in Washington, with special emphasis on waterborne transmission of the disease.

Four successive seasons of aquatic mammal trapping established that Giardia-positive animals are distributed widely throughout Washington state. During the 1977-78 trapping season, 19% of beaver and 43% of muskrat samples submitted were Giardia-positive. Positive animals were identified in 31 of 39 counties, and in several protected watersheds. Positive animals were trapped in the 1978-79 and 1979-80 seasons in the Cedar River watershed.

An analysis of human cases followed (between July, 1978 and March, 1980; excluding migrants, immigrants and non-residents.) It revealed that 44% had consumed untreated water within two months before onset of symptoms, 18% had recently traveled to another country, and 70% of infected children had exposure to a day-care center. (Secondary transmission may account for many of the 79 clusters of cases observed during the project.)

Outbreaks identified during the period of the project include one among Boistfort Water System customers. Eleven positive cases were identified during a five-month period, and a survey revealed that customers had an approximately 20% incidence of giardiasis-like symptoms over the two months. Two control communities surveyed revealed less that 5% prevalence. No cysts were recovered from orlon filter samples of Boistfort water or from beaver stool samples collected upstream from the intake. After an increase in chlorine residuals, no new human cases were diagnosed.

Surveillance for Waterborne Giardiasis: Report of Workgroup 8

Workgroup 8 began with Larry Foster describing a new program being put into place in Oregon: giardiasis reports statewide are going into a central computer, with various benefits to result. (Before, Oregon had no statewide data-retrieval system at all.) The EPA provided Group 8 with the following questions:

- *How can waterborne giardiasis cases be identified?*
- *Should water systems be surveyed and tested for Giardia potential?*
- *How can reporting of giardiasis be improved to identify connections between water systems and giardiasis? Who needs to coordinate with whom in order to make the system work?*
- *Should Oregon undertake a study like Washington's to identify Giardia hotspots?*

The EPA also suggested the following possible final product: A description of the necessary activities in a good surveillance program. (What needs to be identified? How do you identify it?) Ideas for improving the current system for identifying waterborne giardiasis. A brief outline of a possible program for identifying Giardia problem areas in Oregon.

Report of Workgroup 8

The group members found they needed to simplify the problem in order to deal with questions of surveillance only. They therefore defined the following arbitrary assumptions (although everyone agreed that all of these would never happen in real life) simply for the sake of narrowing the issue.

(Inherent assumption:

All surface-water systems are potentially at risk.)

Assumptions:

1. The technology can be put into place.
2. The politics will be favorable.
3. The money will be there.

Surveillance for Waterborne Giardiasis: Report of Workgroup 8 (Continued...)

How do we identify waterborne giardiasis?

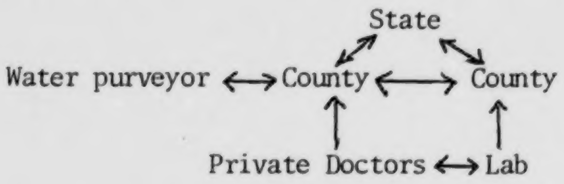
1. Get a clustering of cases.
2. Rule out other possibilities for clusters.
3. Do a formal investigation of:
    - people cases
    - the water system

How do we improve identification and reporting?

1. Require reporting on standard form: develop and implement statewide training for people to fill out forms.
2. Computerize information, to be able to draw out previously inaccessible facts and figures.

Who should coordinate the reporting?

The group concluded the state should coordinate the reporting. They sketched the flow of information as follows:



Should we do a study like Washington?

No; most information would be redundant, and the statewide reporting system will help identify "hotspots."

What goes into setting up a good surveillance program?

1. A ranking system for those at most risk, to determine where first to intervene on a limited budget.

Waterborne Giardiasis and its Epidemiology: Report of Workgroup 8 (Continued...)

2. Education of the purveyor (for consistent results) and of operator (for general good maintenance of systems.)

3. On-site survey of system:

    a) Check records,

    b) Make tests,

    c) Examine site, (etc.)

Ed Lippy presented a talk on Control Measures, and then sent the following information to include in this report.

Giardia Control Measures for Water Systems

(Edwin C. Lippy, P.E.
Health Effects Research
Laboratory, U.S. EPA
Cincinnati, Ohio)

Figure 1 shows giardiasis outbreaks, distributed geographically. Many water systems in the northeast and far west use mountain streams as a source of supply. Because of good-quality water and absence of point-source wastewater discharges in the watershed, they provide minimal treatment normal chlorination only. They are required to comply with the National Interim Primary Drinking Water Regulations which specify microbiological limits for coliform bacteria. Chlorination provided in these situations is effective in destroying the coliform organism, but is ineffective in inactivating Giardia cysts.

Figure 2 presents deficiencies in public water systems that cause and contribute to giardiasis outbreaks. Deficiencies are coded by number in the figure according to five categories: those that used untreated surface water, those that used untreated ground water, those with inadequate or interrupted treatment, those with deficiencies in their distribution network, and those with problems of miscellaneous or unknown cause.

The first two categories need no further explanation. Category 3, "Inadequate or interrupted treatment," is defined as breakdowns or defects in the treatment process that permit inadequately treated water to be distributed. Category 4, "Deficient distribution network," includes events that cause contamination through such problems as cross-connections, ruptured mains or open resources. Category 5 includes causes not included in the other four categories, or the outbreak was not investigated enough to determine a cause.

Figure 2's results show that two-thirds of giardiasis outbreaks are caused by not providing adequate or reliable treatment. Category 3 was further subdivided to highlight treatment deficiencies, as shown in Table 1. Chlorination deficiencies contributed to 28 of the 35 outbreaks, and clarification deficiencies (coagulation, settling filtration) were responsible for the remaining 7.

The two most common chlorination problems that contribute to outbreaks are: 1) dependence upon a microbiological standard that does not indicate "safe water," and 2) failure to apply currently available technology.

The coliform standard or microbiological contaminant level has been used since 1914, perhaps attaining an unjustified significance. No sound scientific basis exists for its use as an indicator of safe water. Rather, it indicates contamination, and should be interpreted accordingly. A positive coliform sample doesn't mean the water is unsafe to drink. Nor does a negative coliform sample! A positive sample does mean that coliform bacteria are present. It may mean that pathogenic or disease-causing bacteria are also present. A negative sample indicates the absence of coliform bacteria. Pathogens including parasites, virus and bacteria may still be present. Where water systems may be challenged by Giardia cysts, reliance should not be placed totally upon

Giardia Control Measures for Water Systems (Continued...)

meeting the coliform MCL, but should be placed instead on treatment measures capable of offsetting the challenge. Minimal chlorination is effective in destroying coliform bacteria to comply with the MCL, but cysticidal dosing requires greater amounts of chlorine, and longer contact times.

To effectively inactivate Giardia cysts, chlorination must be practiced accounting for pH, contact time, temperature and turbidity. Chlorine must be dosed in response to plant output, and the facilities should be reliable to the extent that the process is not interrupted.

The pH of the water being chlorinated is extremely important: it determines the percentage of hypochlorous acid and hypochlorite ion in solution. At low pH, the hypochlorous acid form predominates. At the higher pH, the hypochlorite ion is dominant. Hypochlorous acid is about 100 times more efficient in killing power. (The pH/chlorine relationship is shown in Figure 3.)

Temperature must also be taken into consideration, since chemical reactions progress more rapidly in warm water. Sufficient contact time is necessary to allow chlorine to do its job.[1]

The interference of turbidity with chlorination is not as well defined as the relationships of pH, temperature, and contact time. Interference occurs when turbidity exerts a chlorine demand. This thereby reduces the chlorine available to act as a disinfectant or cysticide. Interference also occurs when particulate matter entraps cysts within the particle structure and shields the cyst from the disinfectant.

Turbidity has less influence on chlorine demand than was originally thought. A recent study showed that less than 10% of the chlorine demand was exerted by particulate matter when the turbidity was at a concentration of less than 20 NTU's. The chlorine demand, amounting to greater than 90%, was exerted by the dissolved and colloidal matter that passed through a 0.45 micron filter. This should not be used as an excuse for failing to provide filtration where needed, but should emphasize the need for adequate disinfection as a necessary treatment step in the multiple-barrier concept.

Reliability in the chlorination process should encompass all factors that assure continuous, uninterrupted and adequate disinfection. Facilities that feed chlorine should be able to respond to changes in plant output. The demand for water in a community fluctuates from highs around 8:00 to 10:00 a.m. and 8:00 p.m., and lows between midnight and 6:00 a.m. Constant-feed chlorinators paced to dose for an average output will underfeed during peak hours and overfeed during low-demand hours. (This is especially noticeable during the early morning hours: people turn on their taps in the morning and get a blast of chlorine-laden water.)

Reliability in chlorination also requires two cylinders yoked to the chlorinator so that when one cylinder is empty another can be put in to use without interrupting feed. Current technology provides for automatic switchover from the empty to the charged cylinder. Automated monitoring devices are available to give continuous readout of chlorine residuals. Auxiliary power should be provided so chlorine feed isn't interrupted by a power failure.

Giardia Control Measures for Water Systems (Continued...)

Emergency power generation facilities with automatic start-up are available and should be incorporated in the chlorination facility.

Control measures implemented during actual giardiasis outbreaks have been published, and are listed below.[2]

Generally, control techniques used in outbreaks consist of immediate steps taken to control disease, and recommendations for permanent long-term improvement to the water system. The immediate steps usually improve chlorination to the extent that a cysticidal dose is applied. The long-term measures generally require structural improvements, which means 3-5 years' lead time for design, financing and construction. Therefore, it is important that the immediate steps taken to control outbreaks are sound and reliable.

[1] The relationships between time, water temperature, and chlorine are demonstrated in the article, "Effect of Chlorine on Giardia lamblia Cyst Viability," Applied and Environmental Microbiology, February,1981, pp. 483-487.

[2] Control measures have been published in the following articles:

"Tracing a Giardiasis Outbreak at Berlin, New Hampshire," Journal of American Water Works Association, Vol 70, September, 1978, pp 512-520.

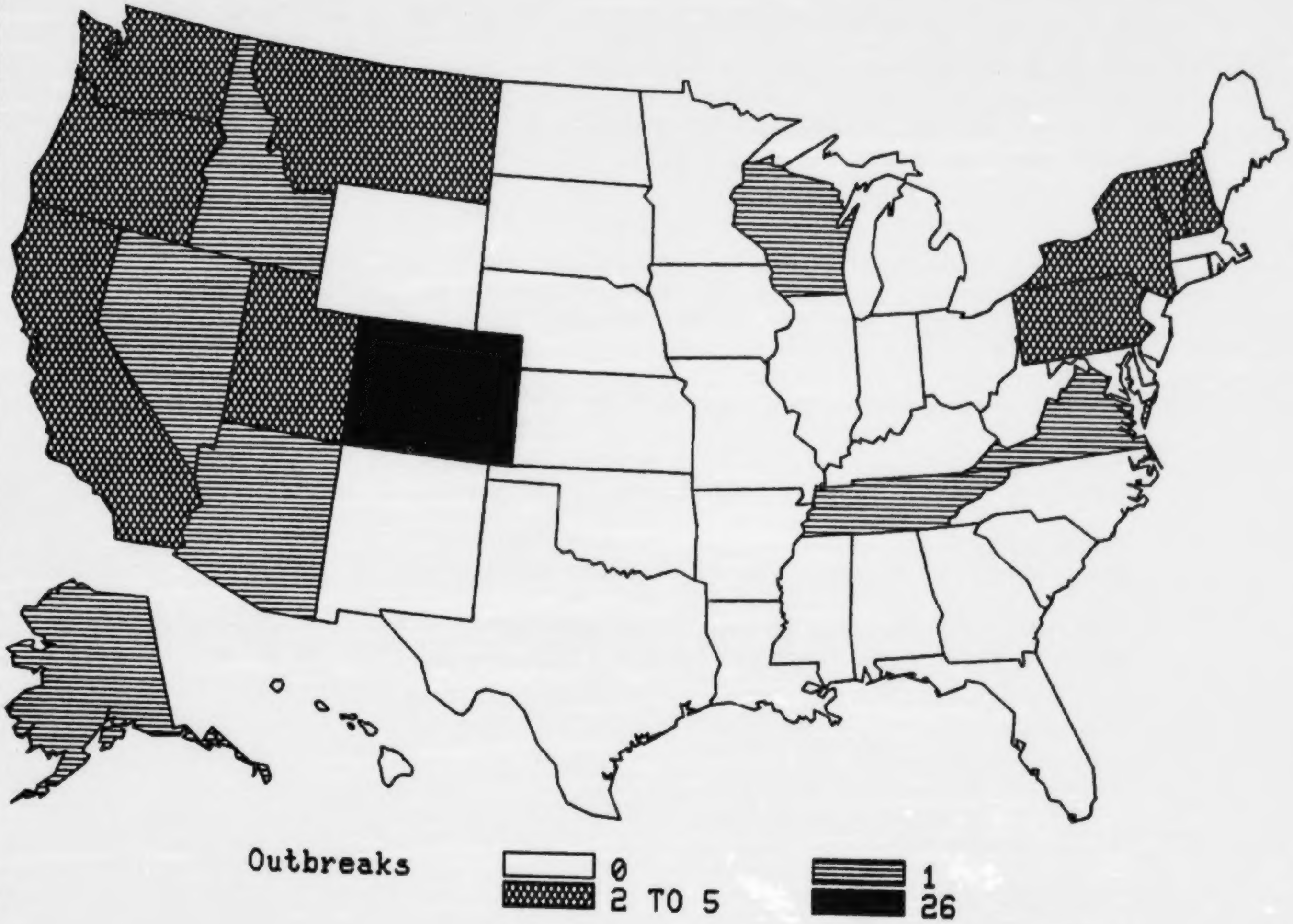
"Waterborne Disease: Occurrence is on the Upswing," Journal of American Water Works Association, Vol 73, January 1981, pp 57-62.

"The Role of Filtration in Preventing Waterborne Disease, " Journal of American Water Works Association, Vol 74, December, 1982, pp 649-655.

Giardia Control Measures for Water Systems (Continued...)

Figure 1:

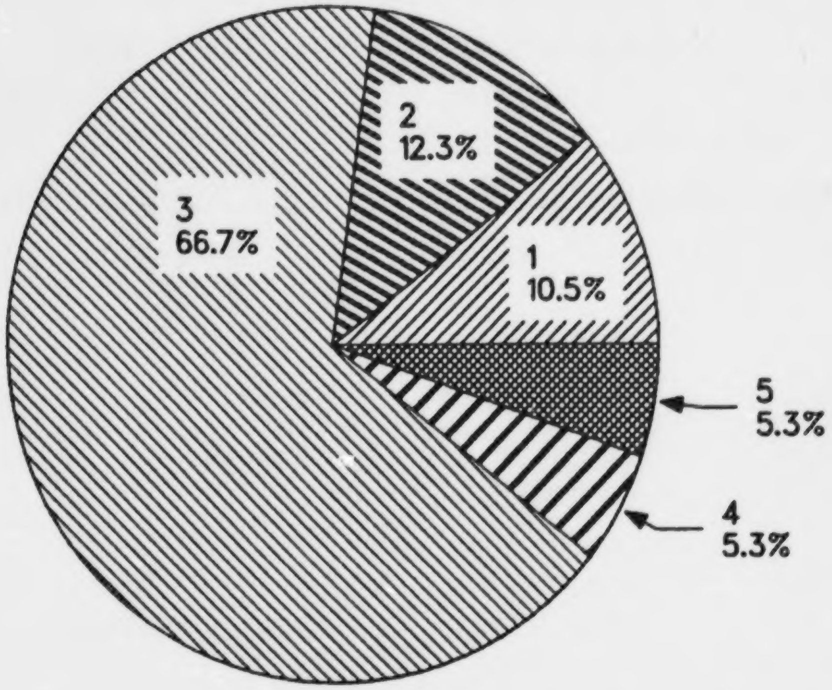
Giardiasis Outbreaks, 1965 to 1982: Geographic Distribution


Outbreaks
0
1
2 TO 5
26

Giardia Control Measures for Water Systems (Continued...)

Figure 2:

Public Water System-Related Giardiasis Outbreaks, 1965-1982



1 - Used untreated surface water
2 - Used untreated ground water
3 - Inadequate or interrupted treatment
4 - Deficient distribution network
5 - Miscellaneous or unknown causes

Table 1

GIARDIASIS OUTBREAKS
PUBLIC WATER SYSTEMS, 1965-1982
INADEQUATE/INTERRUPTED TREATMENT (#3)

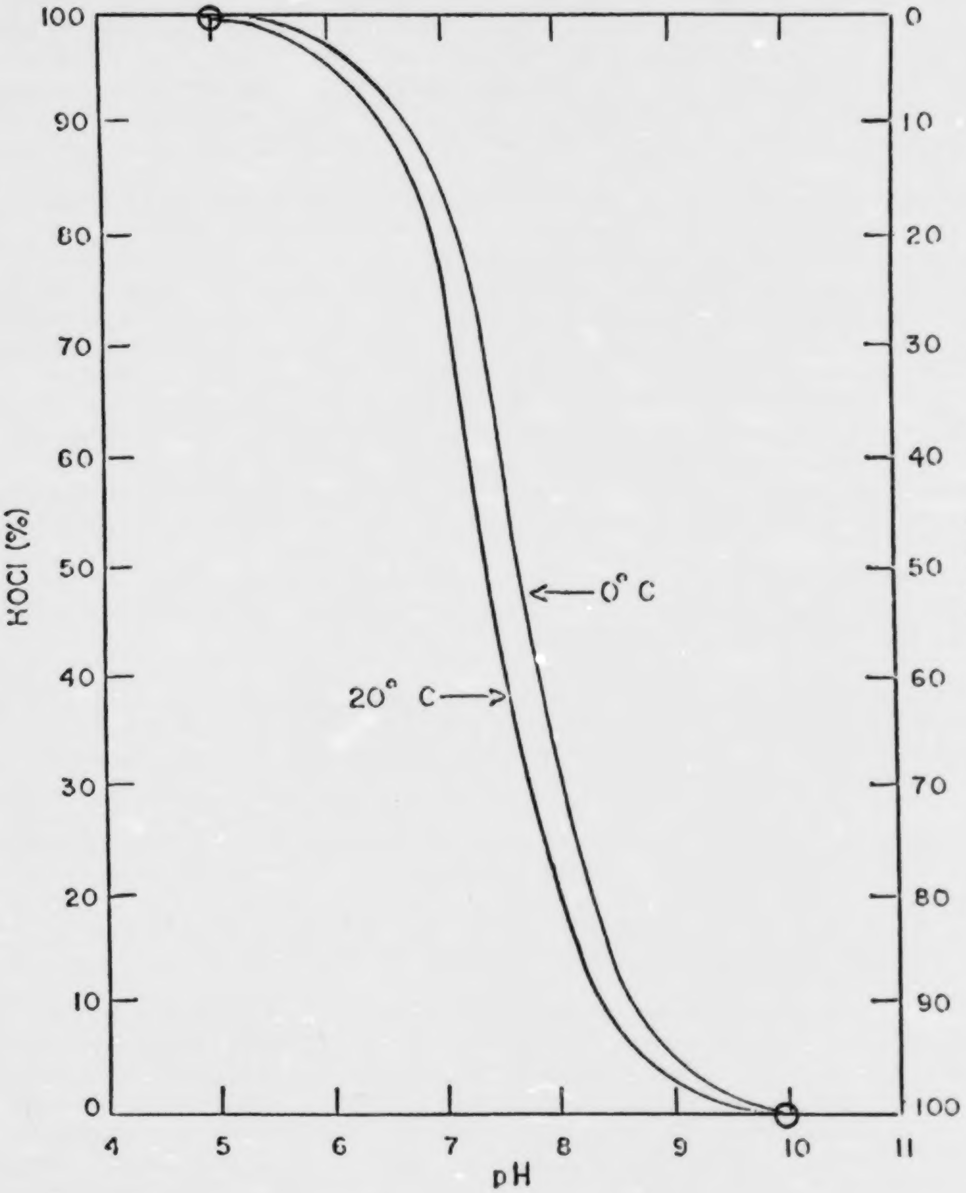
DEFICIENCY

CHLORINATION (28)			CLARIFICATION (7)	
INOPERATIVE	INADEQUATE	UNKNOWN	COAGULATION	FILTRATION
3	13	14	5	4
			CAMAS	CAMAS
			BERLIN	BERLIN
			VAIL	LEAVENWORTH
			ESTES PARK	LARIMER CO. (BYPASS)
			PAGOSA SPRINGS	
RENO				
FLORIDA RIVER ESTATES				

Giardia Control Measures for Water Systems (Continued...)

Figure 3:

Effect of pH on quantities of hypochlorous acid (HOCl) and hypochlorite ion (OCl^-) present in water (Berg, 1966)



Operation and Maintenance Problems Implicated in Giardiasis Outbreaks

(John Kirner
City of Tacoma Water Division
Tacoma, Washington)

Camas and Leavenworth are both cities that have suffered from a waterborne outbreak of giardiasis. I'd like to talk to you from two standpoints today. First, what went wrong, and second, a more general basis, what can go wrong, and what can we do to keep it from happening?

In evaluating a water system, I think the first two things I'd say is 'survey, survey.' Know what you've got. If you don't know what you've got, you're never going to be able to find the source of the problem. Don't just look at the plans; don't just listen to what the operator tells you. (He probably heard it from the guy who was the operator before him, and has never bothered to dig all the way through and follow each line through the plant to see where it goes.)

Try to understand what the source is. Dr. Juranek talked about walking the supply at Camas yesterday: when he first walked the supply he found sticks that had gnaw marks on them. This was the first time anybody had bothered to check out the beavers. In Leavenworth, for another example, we had two supplies coming into a chlorination system. 148 people fed off a raw water line. (They should have been goners.) Then it went through a water treatment plant with a filter bypass plant. This is something to look for if you're involved in public health inspection of new facilities. a filter bypass plant is something you always want to try to avoid. (In some situations, the argument will be, it's absolutely essential for emergencies. Tell them to put it in, but leave a piece of pipe missing. If they have an emergency they can put the pipe in, but it's not necessary all the time.) Valves are not adequate protection.

Then, in Leavenworth, water was distributed from the treatment plant to the reservoirs through pump stations and in some cases through automatic valves, and others in the well from the other end.

I don't share the total concern over pressure filters that some other engineers have expressed. The reason I don't share quite that level of concern is I think there are some small communities working in mountainous terrain where they may be the best thing you can get.

Most of you are familiar with rapid-sand filters and know that a surface washer suspended 13 or 9 inches above the media is about as effective as no surface washer at all. Normally you try to get the surface washer to within a couple of inches of the media, so when it does clean the system it provides some break-up of the material that's collected on top of the filter.

When these things are "built," (what putting the media into a filter is called) they have about 18 inches of anthracite media. A conventional rapid-sand filter, as constructed today, will almost always have a layer of this anthracite coal above some silica sand, possibly above some garnet sand. The anthracite coal is coarse. It's constructed so, in a cross-section, the filter has coarse material, finer material, finest material. The idea is that you can get longer filter runs and hold more material in the filters by allowing it to penetrate

Operation and Maintenance Problems Implicated in Giardiasis Outbreaks (Continued...)

this coarse material. In theory, nothing would get through the finest material. (If particles that you are attempting to remove by filtration get to the finest material, they will mat out, not penetrate, increase head loss in the filter, and require a backwash.)

In Camas, we regraded and replaced the filter media. The problems we saw were definite media loss, primarily in the anthracite: not an unusual occurence. (Most utilities with rapid-sand filters do replace anthracite media on a regular basis.) The interesting thing about the Camas operation is that the plant was built prior to the Safe Drinking Water Act, when the 1962 public health service standards called turbidity an aesthetic concern rather than a health-related concern. They built the plant like that just because they didn't like mud in the water.

The media they put in should have been effective, but the problem is one of pre-treatment. I don't know how many treatment plants I've looked at over the years that have had little or no pre-treatment. Pre-treatment can be any number of chemical additions, depending on the situation you've got. Great pre-treatment can be the addition of some polyelectrolyte primary coagulant and a shot of filter aid with ten minutes' detention time before the filter, or it can be a whole battery of chemicals, an hour of mixing and six hours of settling prior to filtration. What you have to do depends on what you have to deal with. And it's gotto be evaluated case by case. But one thing you can be sure of, if you have a sand filter and you don't add chemicals, you will pass particles the size of bacteria, Giardia, and viruses.

Let me give you an example: Giardia bacteria are particles in the .3 to 4.0 micron size range. If you look at a rapid-sand filter, the smallest sand gradation is going to have an effective size of maybe .25 but more likely .35 to .5 millimeters, or 350 to 500 microns. So you're talking about a field of boulders from which you are trying to remove some pebbles. Obviously there's a lot more to filtration than simple straining.

Where some ion exchange is taking place there is some increase of size and floc formation and then removal. There's some absorption taking place. You can't filter in the conventional public health sense unless you provide adequate pre-treatment and you design that pre-treatment to deal with the problem at hand.

At Camas, they were adding about 10 to 12 parts per million of alum, whether they needed it or not, 365 days a year. It was probably not doing a darn thing, because they had all of about 10 minutes of detention time after an addition of alum. Obviously, standby chlorination should have been provided.

If you're using alum, you could monitor for aluminum ion, and if you get a significant passage you obviously are not forming the floc and removing it in the filter.

Operation and Maintenance Problems Implicated in Giardiasis Outbreaks (Continued...)

The turbidity blip that comes through a filter after backwash is something we were concerned about. We installed a plant bypass to waste and found a number of little cross-connections. (You'd think that no one should be more sensitive to this than a water utility.) It goes back to the period of design: turbidity was not considered a health-related concern. The plan was to remove the big chunks, and if it had a few little cross-connections, no one worried.

The Leavenworth watershed is in the Alpine Lakes Wilderness Area, one of the most beautiful pieces of terrain on earth, and also one of the heaviest-used areas by backpackers. The water is taken from Icicle Creek. The parking lot to get people up into this wilderness area is adjacent to the water treatment plant. They maintained a chlorine residual of sorts at Leavenworth: .3 to .4 parts per million. Detention time was fairly short: 10 or 15 minutes. Certainly not adequate by what we consider the situation to be now. Their filter plant was a gravity filter plant, improperly operated. They had chemical feeders, the ability to feed two primary coagulants, were set up for alkilinity addition, plus alum, plus filter aid. The tanks had never been used. They were bone dry, sitting down in the basement. Fortunately, the feed equipment was there: all we had to do was fire it up. Their raw water turbidity was about 1.2 and their treated water turbidity about .9, with no pre-treatment. What that means is that they removed about 25% of the material coming in. After not being able to bring that turbidity down despite a lot of work with pre-treatment, we got down into the filters and started scratching around. This is where we discovered that the majority of the media had left. It was replaced by some silt and organic material that made the level in the filter beds look reasonable, but it was just muddy. (You could take a sample up to the lab, put it in a jar, add some water, shake it up and it would look like coffee.) In this case, they were inadequately cleaning the filter media by inadequate backwashing.

At Camas we had a failure in disinfection , and a failure in pre-treatment. The question was asked about the support media in the pressure filters: the media down to the fine garnet was level and undisturbed. There was some mounding in the coarse garnet beneath the fine garnet but it should not have led to a problem. In theory, if you're intact through your fine-garnet media, you should have had a layer there that could have done the job, given adequate pre-treatment.

At Leavenworth, there were problems with disinfection, pre-treatment and backwashing. It's possible, by failure to operate properly, to even wipe out multiple barriers.

Camas is about 6,000 people, which is still a small utility. Leavenworth is about 2,000-3,000. Camas actually had a fairly good staff that we were later able to give some instruction and feel reasonably confident that they'd be able to operate the filter plant properly and do a good job of it. At Leavenworth, I don't think we ever felt comfortable that they were going to do a good job with the operation and maintenance of that system. You're not going to find utilities of that size that can put a man in the plant for the full time of operation. That's a key, because the raw water is not going to stay the same. This means the requirements for pre-treatment will vary.

Operation and Maintenance Problems Implicated in Giardiasis Outbreaks (Continued...)

I'd like to suggest a couple of ways to help, if not make the system right, at least to improve your odds. I mentioned the problem of pre-treatment with alum. It's a careful balance of alum, alkilinity, temperature, and pH, and the odds of staying in balance during a rainstorm in a small watershed are practically nil. I'd like to suggest that you avoid alum as the primary coagulant on these small utilities. Take a look at the possibility of using a polyelectrolyte as the primary coagulant and back that up with a filter aid. The polyelectrolyte primary coagulant seem to be less sensitive to changes in water quality than alum. They are more expensive, but a lot less expensive to a water utility than a waterborne disease outbreak. They require less operator skill.

Another thing I think needs more emphasis is the use of disinfection in the control of these outbreaks. I noticed yesterday when somebody brought up the question of disinfection to control Giardia, people on both sides of me said you can't do it. And yet we're hearing that disinfection levels in the 1.0 to 2.0 mg per liter free residual at proper pH's, at proper temperatures, at proper detention times, can provide a fairly good level of protection. Let me throw out another possibility: if a utility doesn't have proper detention time to allow a 1 mg per liter - 2 mg per liter dose, give some thought to chlorinating at a higher dose, and then de-chlorinating.

The question of the effect of turbidity on disinfection was brought up. I think the EPA has done some very good research on turbidity effects, which is why we have a turbidity limit as a health-related parameter. Turbidity in some forms exerts a strong chlorine demand, provides shelter for bacteria and provides precursors for triahlomethane formation. In some of the utilities we looked at, though, particularly when you're looking at a mountainous region, you may have inorganic turbidity that doesn't cause problems. You may be able to chlorinate at substantial levels without encountering some of the problems normally associated with turbidity.

Reliability: make the thing reliable. Bob Willis from Portland made the comment about the difficulty of getting the residual analyzed or getting a loop system that worked properly. You're not going to be able to sell a small utility on using that stuff anyway, even if it did work properly. But what you can sell them on at a reasonable price is at least the use of an automatic changeover yoke on a couple of cylinders, that when the guy doesn't go up and check the cylinders except for a couple of times a week, at least there will be some chlorine running into the system, or a good chance of chlorine running into the system.

I think there's enough information from Dr. Meyer's work and from work at EPA for you to get a pretty good idea of doses of chlorine that would be required under different conditions to provide some protection in areas where you don't have filtration.

Colorado Case Study

(Rick Karlin, P.E.
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Background

During the mid-1970's the Colorado Department of Health became increasingly alarmed about the occurrence of Giardiasis among residents and visitors to the State. Although most of the infections were (and still are) associated with day care centers and consumption of untreated water by bikers, skiers and the like, concern over the vulnerability of consumers of public water supplies began to mount.

Data available at that time indicated that extreme time-concentration factors were necessary for the deactivation of Giardia lamblia cysts. This coupled with the strong association between surface water and Giardiasis led to the conclusion that filtration of all surface water is necessary to adequately protect the public from Giardiasis.

Regulatory History

With the advent of the Federal Safe Drinking Water Act in 1974, and subsequent EPA regulations, Colorado was required to significantly alter their drinking water regulations. (Regulations went from a 2 sided single page to 88 pages in length). New "Colorado Primary Drinking Water Regulations" adopted in 1977 changed the pre-existing disinfection requirement (imposed on all public water supplies) to include the statement "Surface waters shall be disinfected by both chemical and physical treatment, including filtration." This statement was added principally to assure the removal of Giardia cysts and other particulates capable of causing disease or of masking pathogens from chlorination.

To assure that the "filtration" message was not lost, "Disinfection" was defined as "the effective killing or removal of pathogenic organisms from public water systems by means or methods utilizing chemical and physical treatment, including filtration and related processes such as coagulation and sedimentation."

Colorado Case Study (Continued...)

The "Disinfection" definition was added in a last minute effort to assure filtration was properly "defined" and required. As with most last minute crunch efforts, some important details were overlooked. Principally, the definition of disinfection, if literally interpreted, required filtration of all water (including ground water). Secondarily it described filtration and "related processes such as ..."without directly requiring chemical pretreatment and sedimentation. Research and plant data have long indicated that particulate removal is quite poor in granular filters in the absence of chemical pretreatment. Therefore, the 1977 regulations, while attempting to require the proper filtration of all surface waters had somewhat missed the mark.

The 1981 revision of the Colorado Primary Drinking Water Regulations (brought about by the EPA regulation of trihalomethanes, sodium, etc.) attempted to address the shortcomings of the "first generation" filtration requirement.

All systems were required to disinfect and surface water suppliers to filter as before. However, the definition of "Disinfection" was changed to "the removal of pathogenic organisms from public water supplies by chemical and physical treatment, including, for surface waters, filtration and related processes such as coagulation and sedimentation."

To assure that a proper understanding of what is involved in the filtration process if maintained in the regulation "filtration" is specifically defined to mean "the physical and chemical process for separating suspended and colloidal impurities from water by addition of chemicals, sedimentation, and passage through a porous medium." An excellent definition of conventional high-rate filtration process (if I do say so myself). As indicated earlier, "filtration" without adequate pretreatment is inadequate to assure the removal of Giardia cyst size particles.

It appeared that all loopholes had been closed and flaws corrected. All public supplies were required to disinfect and all surface supplies to filter. However — in our zeal to properly define "filtration" we had technically eliminated alternative filtration techniques. Specifically neither diatomaceous earth (D.E.) nor slow sand filtration generally lend themselves to chemical pretreatment. (Coagulation gums up the works for both of these processes). Direct filtration does not include a sedimentation step.

Colorado Case Study (Continued...)

In practice, we have approved what we feel are properly applied "non-conventional" filtration techniques. (D.E., slow sand and direct filtration). We anticipate a third generation regulation more clearly defining what pretreatment/filtration techniques will be acceptable.

Confirming Data

The appropriateness of the filtration requirement has been confirmed by waterborne Giardiasis data collected over the last 18 years. Interestingly most of the data has been collected since the institution of the filtration requirement.

In Colorado we have had 15 confirmed waterborne giardiasis outbreaks associated with public water supplies. The time distribution of these outbreaks is worthy of discussion specifically: 1965-1979 four outbreaks, 1980-1982 six outbreaks, 1982-present five outbreaks. The most notable feature of this data is the large increase in documented outbreaks beginning in 1980 (from 0.28 per year from 1965-1980 to 3 per year from 1981 to 1982). We feel that this apparent increase in outbreaks is in fact due to an improvement in our investigation and reporting procedures.

Specifically, during this period (1980-1982) we were conducting intensive waterborne disease surveillance under a special grant from the USEPA and Center for Disease Control. The grant was given to several states to determine whether more intense surveillance results in a higher rate of documented disease and what surveillance methods appear to be most effective. The specific results of this study are available as a separate report, however, a few highlights are appropriate for mention here.

During the study period a full time waterborne disease "investigator" was added to the Department staff with the full time assignment of follow-up of waterborne disease reports. Initially, telephone surveys were conducted whenever an unsafe bacteriological sample (exceeding coliform maximum contaminant level) was received. No outbreaks (due to Giardia or other agent) were detected in this manner.

Colorado Case Study (Continued...)

Lack of "success" via this approach led to utilization of a different technique, namely the follow-up upon reported potential waterborne disease. Disease reports from citizens, local health authorities, medical communities were investigated via phone surveys, where appropriate, to determine the nature, extent and apparent cause of enteric disease within a community. Whenever public water supplies were implicated in outbreaks, engineering investigation as to treatment deficiencies or breakdowns which may have led to the apparent outbreaks were performed. Where appropriate, enforcement actions were taken to assure correction of deficiencies and prevent recurrence of outbreaks.

At the close of the study period, we decided to maintain the surveillance program, and it is in place currently. Although the program has apparently "increased" waterborne disease occurrence in Colorado, it has also enabled us to identify and correct a number of deficient water systems.

Review of the engineering aspects of all fifteen giardiasis outbreaks revealed the following common features:

(1) Fourteen of the fifteen outbreaks were associated with surface water supplies.

(2) None of the outbreaks occurred on a system equipped with full treatment. Full treatment includes coagulaton/flocculation, sedimentation, filtration and disinfection.

(3) None of the outbreaks was associated with violation of the Colorado Primary Drinking Water Regulations/USEPA turbidity standard (1.0 NTU).

(4) None of the outbreaks was associated with a violation of the Colorado Primary Drinking Water Regulations/USEPA bacteriological standard.

From these facts it can reasonably be concluded that meeting the drinking water standards alone will not assure prevention of Giardiasis, and in fact that only proper installation and operation of full treatment on all surface water supplies can reasonably be expected to prevent waterborne giardiasis outbreaks.

Colorado Case Study (Continued...)

Enforcement Results

Inventory records in 1981 showed 25 surface water systems in Colorado which lacked complete treatment, inspections in the meantime have revealed five more, for a total of 30 systems failing to meet the filtration requirement. Of these, 21 have plants under construction as a result of either persuasion or coercion. "Coercion" in this case means issuance of a legal Notice and Order to construct facilities. All systems serving 500 or more consumers are now treating or constructing treatment facilities.

There has been little "resistance" from the engineering community or from the regulated community, in general, to the filtratin requirement of the Colorado Primary Drinking Water Regulations.

A large portion of surface supplies are associated with the tourist industry. The resort and other suppliers recognize the potential damage which they may incur should they fail to provide a safe water supply to the public. This coupled with a fairly widespread educational effort has resulted in a high level of cooperation from water purveyors.

In summary, we feel that the Colorado filtration requirement is technically sound and public health effective. Its implementation has been successful and its long-range effect should be the reduction of waterborne disease.

Water-System Evaluation/Risk Evaluation: Report of Workgroup 5

The EPA gave Workgroup 5 the following suggested discussion questions:

- *How do you determine the risk associated with a water system? What physical and chemical characteristics of a water supply create a risk of giardiasis?*
- *Can we realistically decide what risk is associated with finding, for example, one Giardia cyst in a drinking-water supply?*
- *Should viability of cysts be considered in determining risk?*
- *How can water-system operators be informed about risk evaluation and control measures?*

The EPA also suggested the following possible final product: Criteria for determining when a water system is at risk from Giardia. Recommendations about how to inform or educate water-systems operators about assessing their water systems for potential Giardia risk, and about appropriate control measures for Giardia.

Summary:

The group looked primarily at two questions:

1. How do you determine risk associated with a water system?
2. Specifically, what physical and chemical characteristics of a water supply create risk of giardiasis?

To simplify the issues involved, the group listed the following arbitrary assumptions.

- Giardia is ubiquitous.
- Waterborne Giardia is a public-health problem.
- Viable organisms exist in surface waters (two-month survival.)
- Cysts found in water can be infectious to humans.
- Our focus is on Oregon water systems and Oregon priorities.

The group then came out with specific criteria for determining when a water system is at risk for Giardia. Everyone agreed that even with a priority list established, the final determination of which system(s) were at highest risk would rest with the professional judgement of the investigator.

Determining the general risk associated with a water system:

1. What is the water source?
   If surface:
   - Is there human habitation upstream?
   - Is there animal habitation upstream? In what proximity to the water? What animal(s)?
   - Do humans use the upstream area for recreation?
   - Is there logging upstream?
   - Is the watershed protected?

2. Water quality: what are the physical, chemical and microbiological characteristics of the water?

3. What treatment does the system use? Is it reliable? Is it maintained?

4. What population is at risk?

5. Is there historical (epidemiologic) evidence of disease?

6. What is the finished water-quality record?

   - Has treatment been consistently effective? (As determined by such water-quality parameters as total coliform and turbidity testing, biomass removal, fecal coliform, SPC, or Giardia tests.)
   - Has the water had sufficient chlorine residuals on a consistent basis?
   - Are the system's records adequate to determine the effectiveness of the treatment?

7. What is the season?

   - Winter: lower probability
   - Spring, summer, autumn: higher probability

8. Operation and maintenance of the treatment facility:

   - How is the plant maintained?
   - Is the operator skilled? Certified?
   - What training is given the operator?
   - How well does the operator understand what he or she is trying to accomplish?
   - How fast does the facility's staff turn over?
   - How does the facility test its own quality? Does it have a lab? Equipment? Access to either?

Water-System Evaluation/Risk Evaluation: Report of Workgroup 5 (Continued...)

Determining what physical and chemical characteristics of a water supply create risk of giardiasis:

Looking at the importance of the above items (#1 through #8), the group determined priorities for them with respect to giardiasis risk.

Priority:	Risk Factor:
1	Systems that use surface water, or surface-influenced water (Animal and human contamination that can be seen, found or proved makes this priority more obvious, but the risk exists for all surface-related systems.)
2	Systems with no disinfection, or systems with inadequate disinfection (i.e., is the concentration and/or contact time adequate?)
3	Systems with treatment not as efficient or effective as other possible treatments
4	Systems with an epidemiological history of disease
5	Systems with improper or inadequate operations and/or maintenance
6	History of poor water quality

Control Measures for Giardia in Water Systems

Workgroups 6 and 7 were assigned the same topic and the following discussion questions:

- *If a water system has a real or potential Giardia problem, what emergency measures should be advised? What long-term measures? What is the rationale behind each measure?*

The EPA suggested the following final products: Recommended short- and long-term measures for water systems with current or potential Giardia problems. Measures for systems with no treatment, those with disinfection only, and those with full treatment.

Working separately, the groups came up with similar recommendations. Their reports are presented separately below.

Control Measures for Giardia in Water Systems: Report of Workgroup 6

The group began with some general discussion, and the setting of two arbitrary assumptions (in order to simplify the issue.) The assumptions the group set are:

- There is a thorough knowledge of the system, and a risk-assessment has been made.
- By definition, the duration of the emergency is limited to 72 hours.

The discussion included such ideas as freezing, using the hot-water faucet, and other possible emergency measures, but everyone agreed that these were "special-case" measures, not to be bandied about generally for fear of confusing the issue.

The group then divided the treatment measures into the EPA's suggested three categories: "no treatment," "disinfection only," and "full treatment." The measures the group determined for each situation follow.

Control Measures for Giardia in Water Systems: Report of Workgroup 6 (Continued...)

EMERGENCY AND LONG-TERM TREATMENT FOR WATER SYSTEMS WHERE GIARDIA IS A POTENTIAL OR REAL PROBLEM

	Emergency Measures	Interim Measures	Long-term Measures
System with No Treatment	1. Put out boil-water/bottled water notice. Notice should be in force for three days. 2. Communicate to the public what is being done. Provide continuous notice. 3. Implement emergency action plan. Notify appropriate agencies.	1. Explore use of alternate supplies: trucked? inter-ties with other systems? 2. set up emergency-treatment facilities: -Portable filtration -Portable reservoirs -Chlorinators -Batch chlorination -Reservoir and distribution-system flushing 3. Continue public education & information. 4. Identify the source of contamination and develop a plan to deal with it. (Include as "sources" operation/maintenance problems or a system operating over capacity.) 5. Implement interim control measures for licensed facility (as required by state plan.) 6. Set water-conservation measures & implement.	1. Implement long-term plan: -Find alternate source -Install disinfection/contact time/storage/watershed management/operations controls -Go to full treatment 2. Continue public information. 3. Train, educate and certify operators.
System with Disinfection Only	1. Put out boil-water/bottled water notice. Should be in force 3 days. 2. Communicate to the public what is being done. Provide continuous notice. 3. Implement emergency action plan. Notify appropriate agencies and/or manufacturers' reps for CL-2. 4. Superchlorinate to 2.0 MG/L free residual (assuming 60-minute retention, low turbidity and normal temp.)	1-5 above. 6. Refine disinfection based on factors observed at the site and on water-quality factors. 7. Set water-conservation measures and put into effect.	1. Implement long-term plan: -Find alternate source -Go to full treatment 2. Upgrade or improve disinfection. 3. Continue public information. 4. Train, educate and certify operators.
System with Full Treatment	1-4 above. 5. Review operating procedures to improve effluent water quality, and implement revised procedures. (Goal: 0.2 NTU)	1-4 above. 5. Refine disinfection based on factors observed at the site and on water-quality factors. 6. Set water-conservation measures and put into effect.	1. Improve the source. 2. Upgrade and/or expand present equipment. 3. Continue public information. 4. Train, educate and certify operators.

Control Measures for Giardia in Water Systems: Report of Workgroup 7

Workgroup 7 approached the same topic as Workgroup 6, but from a slightly different outlook. Instead of dividing the treatment choices into "emergency, interim and long term," the group used "short-term" and "long-term."

In developing the chart on the following page, the group considered efficiency and efficacy, what really works, relative cost, funding mechanisms, simplicity and whether the suggested solution suits the system, availability, owner motivation, and urgency.

After finishing the chart, the group came up with a slogan to keep in mind when dealing with a problem that has caused an outbreak:

> "Find It, Fix It, and Don't Forget It."

Control Measures for Giardia in Water Systems: Report of Workgroup 7 (Continued...)

	Short-term Solutions	Long-term Solutions
Systems with No Treatment	1. Issue "boil water" notice and maintain input to media. 2. Establish the source of infection. 3. Establish disinfection appropriate to the system. 4. Flush until effective chlorine residual level is maintained within the system. 5. Continue monitoring and surveillance until problem is controlled.	1. Perform an engineering evaluation. 2. Notify public health authorities. 3. Educate the community. 4. Select alternate source or/upgrade treatment. 5. Inform and educate legislators and decision-makers.
Systems with Disinfection Only	1. Issue "boil water" notice and maintain input to media. 2. Establish source of infection. 3. Increase disinfection residual in mains. 4. Review disinfection system rapidly to see if there are easily solved problems. 5. Continue monitoring and surveillance until the problem is controlled.	1. Perform an engineering evaluation of the water system. 2. Consider alternate sources or additional treatment. 3. Get consultation, information and/or technical assistance as needed. 4. Seek State-mandated improvement.
Systems with Full Treatment	1. Issue "boil water" notice and maintain input to media. 2. Make a quick engineering review to see if there is an obvious problem. 3. Establish disinfection with an effective residual. 4. Establish a system to handle the media, and to keep the media from scaring the public.	1. Perform an engineering evaluation of the system to determine the problem. 2. If cause is an operational one, educate operators and/or change procedures to reduce risk of it happening again. 3. If cause is "a catastrophe," prepare a contingency plan to handle such an event in future.

THE FUTURE: REGULATORY REQUIREMENTS AND RESEARCH NEEDS

On the final day of the conference, the workgroups concentrated on the future. Two looked at issues surrounding setting regulatory requirements, and two looked at what future research was needed. These reports follow.

Future Regulatory Requirements

Workgroups 9 and 10 handled the "Future Regulatory Requirements" topic. The EPA suggested the following discussion questions:

- *Should Giardia be a regulated drinking-water contaminant?*
- *Is it plausible at this time to consider a maximum contaminant level or a treatment standard?*

Future Regulatory Requirements: Report of Workgroup 9

Current State and EPA Status:

The State has just adopted a rule that:
- requires "no pathogens" in finished drinking water,
- defines pathogens,
- doesn't require testing, and
- could mean that finding any Giardia cyst is a violation of the "no pathogen" rule.

The EPA's status:

The Safe Drinking Water Act requires regulation of substances that pose a hazard to health. Giardia meets that test. Therefore, the EPA has two choices:

1) Set a maximum contaminant level (MCL) for Giardia, and set testing requirements for it, or
2) If good testing isn't available, set a treatment requirement.

In the current rulemaking, a treatment requirement may be the choice. That requirement would probably be to filter surface water, with a state regulatory agency to determine cases in which filtration isn't necessary. (The requirement would probably take into account the costs of "reasonably available technology" it would require.)

Future Regulatory Requirements: Report of Workgroup 9 (Continued...)

Group Consensus:

The group believes that Giardia shouldn't be a regulated contaminant in drinking water. It agrees with the EPA that devising an MCL isn't feasible, so mandatory treatment of surface-water systems must be the option. The group recommends such mandatory treatment take the following into account:

- System size, source and vulnerability to Giardia, and
- Some decentralized decisionmaking process should be set up for making decisions about specific water systems.

The group determined the following considerations an agency which determines adequate treatment should address:

1. The water source: Is there activity in the watershed? Is it animal? Is it human?
2. What is the quality of the raw water? What is the form of the turbidity? What are its levels, and what is the variability? Is microbiological contamination present? At what level?
3. The availability or potential availability of operators with skill enough to perform or operate the selected treatment system.
4. Whether or not the treatment is possible as recommended, given the physical limits of the system:
    - Disinfection -- determine time available, pH, temperature, type of disinfectant
    - Filtration, with appropriate pre-treatment -- rapid sand, slow sand
    - Cost of facilities in relation to communities' ability to finance amortization of capital and annual costs.

THE FUTURE: REGULATORY REQUIREMENTS AND RESEARCH NEEDS

Future Regulatory Requirements: Report of Workgroup 10

Background:

The Safe Drinking Water Act requires regulations for any contaminant that "adversely affects public health," as explained in Workgroup 9's report. Since (despite the question of "how adversely?") *Giardia* undoubtedly does affect public health, the EPA will *probably* regulate *Giardia* levels within the next year. It has two choices of method:

1. To set a maximum contaminant level and sample water, or
2. To determine and recommend a treatment technique.

Although there may be monitoring flexibility in a regulation established by the EPA, probably the requirements wouldn't vary nationwide.

In Oregon, the State Health Division requires "no pathogenic organisms" present in finished water (as of 9/20/83). Routine pathogen sampling isn't required, but the Health Division can require it when it wishes.

Discussion:

The group talked over the fact that Oregon's population will be increasing, and as a population increases, it puts pressure on all finite resources. In the case of water, the probable result will be a move to poorer-quality sources. This makes adequate treatment all the more vital.

Some group members offered the opinion that since the rules are set for a worst-case scenario, it seems that the regulated systems have to spend a lot of money for little benefit.

The group agreed with the EPA's view that an MCL and sampling water were not feasible. Reasons given included: we lack information on the infective dose, sampling and analytical techniques can give a false sense that everything is fine, and determining viability of cysts is costly in time and money.

The real problem in setting and recommending a technique is the fact that a test showing *Giardia* to be absent doesn't guarantee that it won't be absent in the *next day*'s test (i.e., the testing can give false negatives). But even with that shortcoming the problems associated with the MCL approach are so much greater that the group agrees with the EPA that the treatment approach is preferable. Ideas include disinfection, filtration, multiple barriers, et al (as presented in other workgroups), including watershed control.

Future Regulatory Requirements: Report of Workgroup 10 (Continued...)

Trends Future Regulations Should Address:

Given all it discussed, the group brainstormed three major trends it could foresee in the next one to five years that future regulations must take into account. Since "solving for Giardia also solves for other water problems," these trends apply to drinking-water regulation in general, as well as to Giardia issues in particular.

1. Increasing pressure on finite resources (in this case, water) will force moves to sources of lower quality. This, in turn, will force more treatment.

2. Additional efforts to increase tourism and attract new business to the state will make the general public more aware of and supportive of better water (or at least of maintaining acceptable levels). The economics of good water versus the liabilities of bad water will be increasingly important.

3. Continued research will identify new analytical methods and treatment techniques, and/or improve old ones. Therefore, future regulations should be worded in such a way as to allow improved methods to supersede the methods they specify. (In one state, the law was so specific on the method to use that it inadvertently prohibited use of a better method that came along after it was set.) Since laws take time and trouble to change, it's easier to add wording for flexibility when they're being formed in the first place (along the lines of, "until upgraded by new data" or "until made obsolete by a proven, newer method.").

THE FUTURE: REGULATORY REQUIREMENTS AND RESEARCH NEEDS

Workgroups 11 and 12 brainstormed lists of what is needed for future Giardia research. The EPA suggested the final product be such lists, and a notation of what needs the research topics would fulfill.

Future Research Needs: Report of Workgroup 11

The group came up with the following ideas for directions of future research.

- Develop a lab-animal model to demonstrate the infectivity of cysts for comparison with other in-vitro indicators of cyst viability.
- Determine the effect of time and temperature on cyst viability in water. Compare to similar studies of survivability of organism on land (freezing-level survival).
- Determine contact times (and concentrations) that various disinfectants require in water to kill Giardia cysts.
- Need to know which animal species infected with Giardia are important in transmission to humans. Need more information on those animals we suspect are involved in transmission.
- Testing needs:
  - Concentration and processing technique
  - Improve methods for recovering Giardia cysts
  - Improve sensitivity and methods for quantifying level of infection
- Find or develop an effective, simple water treatment.
- Improve methods of recovering organism in human and animal stools.
- Develop test for viability (two stages), and determine whether live organism is capable of infecting humans.
- Determine whether there is an immune component: what is the mechanism of immunity?
- Why do asymptomatic carriers occur?
- Set or improve delivery system for infected wild animals. (Possibly do vaccination of wild animals against giardiasis, as has been done for other illnesses?)
- What is the prevalence or incidence of giardiasis in the United States? Broken down state by state?

Future Research Needs: Report of Workgroup 12

The group identified the following as useful directions for future research. (See also Workgroup 11's report.)

1. More efficient, more sensitive and more economical methods of detecting *Giardia* cysts in water.

2. From a biological standpoint:

   - Methods of differentiating *Giardia* strains
   - Host-specificity studies: are specific hosts affected by specific *Giardia* strains and no other?
   - What animals are reservoirs for *Giardia* cysts?
   - What are alternative indicators of fecal contamination of water?
   - Further encystation studies towards possible new ways to break the cycle
   - Less costly ways (in time and money) to determine viability of cysts found in water
   - Further research on methods for culturing *Giardia*, and their effectiveness

3. From an epidemiologic standpoint:

   - Host-specificity studies (learning characteristics of specificity to allow pinpointing of risk groups)
   - Transmission studies, including cross-species transmission
   - What is the natural history of the disease?
   - More on the immune response: why and how does it come about?
   - More effective methods for diagnosing human infections
   - Surveillance: how do we link cases to the source of infection?
   - Human-treatment methods, chemotherapy

4. Control technology:

   - Water-treatment methods: effective methods of inactivating *Giardia* cysts
     - New methods
     - Studies on treatment of water vs. treatment of people
   - Definitive guidelines for disinfection (chlorine, halogens, ozone, ultraviolet, etc.) and minimum treatment requirements.

   - Less costly technologies of water treatment for small systems

   - Techniques to simplify operation and maintenance of water-treatment systems
   - Recommendations for evaluating efficiency of small-quantity water-treatment systems at point of use.

5. Examination of the question: does the problem *Giardia* poses justify spending more money on research? (It makes large numbers of people sick. On the other hand, it doesn't kill anyone. How vital is more research?)

INFORMING PEOPLE ABOUT *GIARDIA* ISSUES

Workgroup 1's topic was "Public Information." The EPA suggested the following discussion questions:

- Do we need to get information out to the general public, particularly day-care operators, backpackers, or people on water systems known to have potential *Giardia* problems? If so, what do we tell these people?
- Do we need to get information to physicians on *Giardia* problems o side-effects of treatments? What is the best way to get the information out to the physicians and the public?
- Do other groups need information on *Giardia*?

The EPA also suggested the following possible final products: A list of segments of the public that need information on *Giardia*, including what specific information each segment might need. A recommended method to get the information out to these people.

Public Information: Report of Workgroup 1

The group agreed that giardiasis is a major health problem. Once that was agreed, the group identified four basic public-information problems:

1. Identifying community groups to address about *Giardia*,
2. Deciding what to tell them,
3. Deciding how to tell them, and
4. Offering information about what they can do.

The group came up with the following suggestions and conclusions.

1. Identifying Community Groups to Address

The group identified two types of audience for information: "everyone," since everyone is in at least one risk group; and special risk groups such as backpackers, day-care centers, and pet owners.

2. Deciding What to Tell People

A general brochure should be available for the general public. In addition, brochures with more specific information should be available for special risk groups. The matrix on the following page addresses the question of what information should go to what specific audience.

3. How Do We Tell People?

It was the group's consensus that a general brochure, plus brochures addressed to specific groups, are the best way to reach people. Also discussed was the idea that a brochure written by a single agency would be better for spreading the general information than having different agencies write different ones. (Concern was voiced that otherwise agencies might waste energy "re-inventing the wheel.") The suggestion arose that a central, coordinating agency should develop the material. Then local, state, and national groups could disseminate it.

4. Other Public-Information Routes or Approaches

The group also identified the following ways an agency could reach the public with *Giardia*-related information:

- press releases,
- dissemination through special groups, such as the Boy Scouts or Girl Scouts,
- a "*Giardia* Awareness Week,"
- having speakers available to present the topic to public groups.

(See Appendix E for an example of an EPA handout about *Giardia* for backpackers and campers.)

Public Information: Report of Workgroup 1 (Continued...)

General audience	Outdoorsperson	Care Center Parents/Guardians	Care Center Operator	Water Purveyors	Users of Water Systems in which Giardiasis is Occurring	Pet Owners	Health-Care Professionals: Veternarians, Doctors	Homosexuals	AUDIENCE ← TOPIC ↓
X	X	X	X	X	X	X	X	X	What is giardiasis?
X	X	X	X	X	X	X	X	X	What causes giardiasis?
X	X	X	X	X	X	X	X	X	How is giardiasis contracted?
X		X	X			X	X	X	● Fecal-Oral
X		X	X			X	X		- Pets
X		X	X			X	X		- Children
X				X	X		X		● Contaminated water supply
X	X			X			X		● Untreated water
X	X	X	X		X	X	X	X	What are the symptoms of giardiasis?
X		X	X		X	X	X	X	How do I confirm I have giardiasis? (Get medical confirmation)
	*				*				Temporary first-aid treatment
							X		Treatment and side-effects
	X	X	X	X	X				Emergency disinfection
	X	X	X	X	X	X	X	X	Prevention
	X	X	X		o	X	X		● Proper environmental sanitation
		X	X				X	X	● Personal hygiene
		X	X		X	X	X	X	Followup information
		X	X				X		Exclusion

* drink fluids to prevent dehydration
o these people may be carriers, so need to know how to prevent transmission

INFORMING PEOPLE ABOUT GIARDIA ISSUES

Communicating about Giardia-related Issues among Concerned Parties

During the course of working on 'Surveillance for Waterborne Giardiasis,' Workgroup 8 came up with a different topic that related to Giardia: that of communications between various people and groups that are concerned about Giardia-related problems, and how valuable the people in the conference were finding the chance to talk and problem-solve with each other. After they finished their surveillance topic, they came up with the following ideas about communications.

Second Report of Workgroup 8

Problem Statement: General communications among and between all parties affected by waterborne disease could be improved.

Groups and people meant by "all parties affected:"

water purveyors
association representatives
County Health people
the Environmental Protection Agency
laboratories
the Association of Oregon Counties
legislators
the State Health people
Water Resources
Inter-government Relations people
the League of Oregon Cities

Suggested Solution: A yearly forum of all parties.

Purpose: To identify each party's water program direction,
To share what has been done,
To develop consensus answers to the following:
- What are Oregon's main drinking-water problems?
- How can the group unite to get action on these?

To share resources on common problems.

Benefits:

1. Face-to-face contact with the people you're dealing with.
2. Solving water problems for Giardia also solves other water problems.
3. If diverse groups pooled resources, they could save money on programs necessary to all.

When the workgroup presented this information to the entire conference, the presentor challenged the conference to act on this idea by saying, "The only thing better than a fantasy is a memory."

FINANCING FOR WATER-SYSTEM IMPROVEMENTS

Dave Phelps of the Oregon State Health Division presented current sources of financing for water systems. Using his information, the workgroup discussed financing major system improvements, particularly for small water systems. The EPA also suggested the group discuss the merits of informing funding agencies about water systems with serious water-quality problems. How should such funding be prioritized? How should the agencies be informed? Who should carry it through?

The EPA also suggested the following possible final products: Description of weaknesses in current funding mechanisms and how they might be put in place. Suggestions on how to influence funding agencies regarding water systems with serious water problems. An outline of water-quality criteria that should be considered in assigning funding priorities to water systems. A group opinion on whether or not someone should request funding agencies to give a high priority to systems with potential Giardia problems.

Financing for Water-System Improvements: Report of Workgroup 4

The group members determined several problems around the general issue of accessibility of funding for capital improvements and operations/ maintenance of treatment facilities. They then determined a strategy to help ease each problem named.

General Issue: Funding for capital improvements and operation/maintenance of treatments plants is difficult to attain.

Problems surrounding the issue	Strategies
Lack of state and local concern and/or commitment	Develop a way to create general understanding of the problem: . Need to make a firm statement that it is a problem. . See "Public Information" group: More media attention focused on Giardia and possible waterborne aspect.

Financing for Water-System Improvements: Report of Workgroup 4 (Continued...)

Problems surrounding the issue	Strategies
Lack of clear understanding of the *Giardia*/water/money relationship	Need a major public effort: . Research: on other diseases, effective treatment, infection levels, cost/effectiveness studies, and the Giardia/turbidity relationship. . Statistical analysis of the epidemiology of *Giardia*: water/day care/other source
Lack of money for operations/maintenance and training of operators	. Set a mandatory certification program for operators. . Obtain more training money from state, federal, or local-rate-structure sources. . State could require compliance with comprehensive planning rules. . Funding agencies could require long-term fiscal planning.
Funding-agency process doesn't address the needs of the water system very well.	. Make funding priorities consistent among agencies. . Establish a fund earmarked for water.

Asymptomatic carrier Epidemiologically, an individual or member of an animal population that has been infected by a disease causing organism and harbors the organism in its body but does not exhibit symptoms of the disease.

Cl_2 The chlorine molecule. In the gas state, chlorine normally occurs as two chlorine atoms (chemical symbol: Cl) bonded together, hence Cl_2.

Contact time In water treatment, the amount of time that a chemical, particularly a disinfectant such as chlorine, is allowed to remain in contact with the water to be treated. The effectiveness of chlorine disinfection is directly related to the chlorine contact time.

Control Measures In water treatment, steps taken by a water utility or regulatory agency to prevent contamination of the water system or to prevent transmitting pathogens through the water system. Increasing chlorine contact time in the water system is an example of a control measure.

Cost effectiveness study The process by which the specific dollar costs of taking some action are compared to the reasonable value of the result of the action. For example, the cost of adding a new water reservoir for increasing chlorine contact time could be weighed against the value of better health protection for the water system's customers. (As in this example, it is often difficult to assign a reasonable value to the effects of the proposed action.)

Cross connection Links, usually plumbing links, through which it is possible for contaminating materials to enter a potable water system.

Cyst In biology, a capsulelike, membrane covered body. Certain organisms, such as Giardia, form cysts in the resting or dormant stage of their normal life cycles.

Disinfection The process by which pathogenic organisms are killed. Usually, drinking water disinfection is accomplished by some physical means such as boiling or irradiation with ultraviolet light, or by chemical processes such as chlorination.

Encystation The process of forming cysts in the life cycle of certain organisms.

Endemic Refers to something, such as a specific disease, which is prevalent in or peculiar to a particular locality or group. (e.g., giardiasis appears to be endemic among beaver in Colorado.)

Epidemiology The study of rapidly spreading contagious diseases.

Excystation The process of emerging from or passing out of the cyst stage in the life cycle of certain organisms.

False negative In water testing, the occurrence of a negative test result when the material tested for was actually present in the sample. In Giardia testing in drinking water, for example, it is not uncommon for the test results to show that no Giardia organisms were found when, in fact, Giardia organisms may have been present in the sample.

Finished water   In a water system, this refers to water that has been treated and is ready for drinking. (See also raw water.)

Giardia   Short for Giardia lamblia, a flagellated protozoan parasite which inhabits the small intestine of amphibians, birds, and mammals. In recent years, Giardia has become one of the most commonly identified human intestinal parasites.

Giardiasis   The gastro-intestinal disease caused by Giardia infection. Symptons include nausea, explosive diarrhea, gas, bloating, cramps, loss of appetite, and if untreated, loss of weight.

Immune component   A molecule or particle, such as an antibody, which imparts immunity or partial immunity.

MCL   Maximum contaminant level, the maximum permissible level of any contaminant in drinking water. MCLs are set by state and federal regulations for a number of contaminants, such as arsenic, mercury, coliform bacteria, and certain pesticides and herbicides.

Mg/l (and mg/l free residual)   Milligrams per liter, a chemical unit describing the concentration of a substance in one liter of a solvent (usually water). "Mg/l free residual" is the concentration of certain chlorine compounds or molecules used as disinfectants in water.

Mutagen   Any agent that causes a biological mutation, a heritable alteration of the genes or chromosomes of an organism. (also mutagenic and mutagenicity)

NTU   Nephelometric turbidity units, a measure of the cloudiness of water. (See also turbidity.)

Pathogen   Any agent that causes disease, commonly a microorganism such as a bacterium or, in this case, a protozoan - Giardia. (also pathogenic and pathogenicity)

Protected watershed   A watershed which is fenced, patrolled, or otherwise regulated to prevent the introduction of contaminants.

PVA   Polyvinyl alcohol, a laboratory preservative used in Giardia analysis that does not destroy the Giardia trophozoite.

Rapid sand filter   A water treatment unit consisting of a tank; several layers of filter material (media) such as gravel, anthracite coal, and sand; and a system of pipes to deliver water to the filter; remove water that has passed through the filter; and wash the filter. A typical operating rate for a rapid sand filter is 2 gallons per minute per squate foot of filter surface. (See also slow sand filter.)

Raw water In a water system, this refers to water from the water source that has not been treated and is not ready for drinking. (See also finished water.)

Regulated contaminant A drinking water contaminant, such as coliform bacteria, for which state or federal limits or treatment requirements have been established.

Reservoirs Epidemiologically, a group of animals or individuals that carry a pathogen and are capable of transmitting the pathogen to the environment or to other individuals.

SDWA The Safe Drinking Water Act (P.L. 93-523) signed into law by Richard Nixon on December 16, 1974.

Shedding (and intermittent shedding) The process of releasing pathogenic organisms, either continuously or intermittently, to the environment. Typically, refers to beavers or other animals shedding *Giardia* cysts in their feces.

Sixty-minute retention (See contact time.) This refers to a contact time of 60 minutes.

Slow sand filter A water treatment unit consisting of a large shallow tank, a layer of sand usually 2 to 3 feet thick, and a system of pipes to supply water to the filter and to remove the filtered water from the bottom of the unit. A typical operating rate for a *slow sand filter* is 0.03 to 0.05 gallons per minute per square foot of filter surface. (See also rapid sand filter.)

Survivability Epidemiologically, the ability of a pathogenic organism, such as a *Giardia* cyst, to survive in a certain environment. (See viability.)

Teratogen Any agent that causes a malformation during the embryological or prenatal development of an organism. In other words, an agent that causes birth defects. (also teratogenic and teratogenicity)

Testing (for coliform, turbidity, standard plate count, biomass, etc.) In the context of this document, the process of collecting samples of water and analyzing the samples for some specific substance.

Trophozoite A stage in the life cycle of *Giardia* organism. The *Giardia trophozoite* stage normally occurs in the intestine of the host.

Turbidity Commonly refers to the cloudiness of water. Technically, however, *turbidity* is a measurement of the light-scattering characteristics of the water.

Viability The ability of an organism to survive in a given environment. (See also survivability.)

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APPENDIX B:

GIARDIASIS, THE ILLNESS

The following charts support Larry Foster's talk, "Giardiasis, the Illness," which is summarized on pages 8 through 13 .

APPENDIX B: GIARDIASIS, THE ILLNESS

Table 1: Treatment[1]

Drug	Adult Dose	Pediatric Dose
Drug of choice: Quinacine HCL (Atabrine)	100 mg 3x daily for 5 days	2 mg/kg 3x daily for 5 days (Max. 300 mg/d)
Alternatives: Metronidazole (Flagyl)	250 mg 3x daily for 5 days	5 mg/kg 3x daily for 5 days
Furazolidone (Furoxone)	100 mg 4x daily for 7 days	1.25 mg/kg 4x daily for 7 days

[1] From *The Medical Letter on Drugs and Theraputics*, January 22, 1982; 24(601):5-12

Table 2: Side Effects of Treatments

Drug	Side Effects
Quinacrine	Frequent: dizziness, headache, vomiting, diarrhea, Occasional: toxic psychosis, insomnia, blood dyserasias, nail pigmentation, rash Rare: liver damage, seizures, severe dermatitis
Metronidazole*	Frequent: nausea, headache, dry mouth, metallic taste Occasional: vomiting, diarrhea, insomnia, weakness, dizziness, paresthesias, rash Rare: ataxia, encephalopathy, pseudomembranous colitis, neutropenia
Furazolidone	Frequent: nausea, vomiting Occasional: allergic reactions, headache, orthostatic hypotension, hypoglycemia, polyneuritis, drug interactions Rare: Hemolytic anemia in infants under age 1, and in persons with G-6-PD deficiency

*Carcinogenic in mice and mutagenic in bacteria. Not FDA-approved for this use.

APPENDIX C:

CONCENTRATING, PROCESSING, DETECTING AND IDENTIFYING *GIARDIA* CYSTS IN WATER

The following pages contain background information supporting Jay Vasconcelos' talk, "Methods of Testing for Giardia in Water." Please see the summary of this talk (pp. 14 through 16) for further information, and for an outline of the modified EPA Consensus Method.

APPENDIX C : CONCENTRATING, PROCESSING DETECTING AND IDENTIFYING *GIARDIA* CYSTS IN WATER

	METHOD	INVESTIGATOR (S)	RESULTS
1.	Membrane Filtration		
	Cellulosic (47mm-0.45µm)	Chang and Kabler USPHS, 1956	Generally unsuccessful
	Polycarbinate (293mm-5µm)	Pyper, DuFrain and Henry Eng 1982, (unpublished)	Passing 1 gal/min at 10 PSI. 15-1800 gal total
2.	Particulate Filtration (diatomaceous earth, sand, etc.)	Shaw *et al*, 1977 Juranek, 1979	Generally good removal but poor eluation
3.	Algae (Foerst) Centrifuge	Holman *et al*, 1983 DHHS, Washington	Good rapid recovery, but limited in field use
4.	Anionic and Cationic Exchange Resins	Brewer, Wright State UN. (unpublished)	Generally unsuccessful
5.	Epoxy-Fiberglass Balston Tube Filters (10"-8µm)	Riggs, CDHS Lab, Berkley, CA (unpublished)	Overall recovery 20-80 percent
6.	Microporous Yarnwoven Depth. Filters (7 and 1µm orlon and polyprolylene)	Jakubowski, Erickson, 1979 and 1980, EPA-Cincinnati	Recovery 3-15 percent Extraction ave. 58 percent
7.	Pellican Cassette System	Millipore Corp. (unpublished)	May be useful for processing filter washings
8.	Filterwashing Apparatus	DuWalle, U. of Wash., 1982 (unpublished)	Claims 75 percent recovery from orlon filters

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TABLE 1

APPENDIX C: CONCENTRATING, PROCESSING, DETECTING AND IDENTIFYING GIARDIA CYSTS IN WATER

PRIMARY CONCENTRATION AND PROCESSING METHODS

1. MEMBRANE FILTER (MF) METHODS

   a. Celulosic (mixed esters of cellulose)

      1. Chang and Kabler in 1956
         First to use MF for cyst recovery. Recovered 20-42 percent at cyst concentration of 3, 5, and 10 cyst/gal. - no cyst found at 1 cyst/gal.

      2. Method was used in 1965 Colorado outbreak (Moore, et al, 1969) using 2 liter size water samples from 10 sites. No cysts were detected. Use of cellulosic filters have generally not been successful in demonstrating cysts in drinking water.

   b. Polycarbonate (PC) Filters

      1. Luchtel and Colleages in 1980 used 293 mm, 5.0 μm pore size nucleopore (PC) filters to concentrate formalin-fixed. G. lamblia cysts from 20 L tap water samples. Recovery rates of approximately 75 percent were reported.

      2. Pyper of DuFrain and Henry Engineers claim good recovery with same nucleopore filter at a flow rate of 1 gal./min., not over 10 PSI, passing 15-1800 gal. in just over 24 hours.

   c. Even with these claims by Pyper and Luchtel, the MF Method has only once (Aspen, 1965) been successful in demonstrating cysts in water--probably because:

      1. Inability to process a sufficient volume.

      2. Inability to remove cysts from filter.

      3. Cysts weren't present at time of sampling during or after outbreak.

2. PARTICULATE FILTRATION

   a. SAND - CDC (Shaw, 1977) used high-vol filtration through swimming pool sand filter (280,000 gal. total over 10 days) - was backflushed into 55 gal. drums and coagulated w/alum. Concentration fed to beagle puppies and after treatment (cheesecloth to wire screening to 30 μm MF to centrifuge) was examined microscopically. First time cysts observed in water supply after concentration.

   b. Diatomaceous earth (DE) - CDC (Juranek, 1979) used DE to remove cysts from seeded water. Problem was that cysts couldn't be removed from DE particles. Brewer (1983) claims 5.2-31.1 percent recovery from DE backwash. Retention through 3 forms (celite 505, HyFlo-Supercel and celite 560) at cyst concentration ranging from 6-16,000 cyst/L. Recovery range between 66-100 percent.

3. ALGAE CENTRIFUGE

   a. Was found to recover more cysts (10X) than a series of MF-filters and nylon screens: 5 vs. 1 day by MF.

   b. May be impractical in field because of power requirement.

   c. If used in lab, 1 large single sample collected in the field could miss cyst.

   d. May find application for concentration cysts from orlon filter washings.

4. ANIONIC AND CATIONIC EXCHANGE RESINS (Brewer - unpublished)

   a. Based on hypothesis that cysts could be attracted to charged surfaces, cysts have a charge of approximately 25mV at pH 5.5 which increases in electro-negativity as the pH rises to 8.0.

   b. Charge attraction techniques have been used for concentration of both bacteria and viruses in water.

   c. Five exchange resins were tested:
      (1. 49 percent recovery from anionic Dowex 1-XY columns
      (2. 38 percent recovery from cationic Dowex 50W-X8 columns

   d. Compared to parallel tests w/diatomaceous earth, exchange resins less efficient in retention.

5. BALSTON EPOXY-FIBERGLASS TUBE FILTERS

   a. Riggs of CSHD, Viral and Rick. Lab., can filter 500 gallons drinking water thru 10" - 8 μm Balston tube filter.

   b. Backflushes w/1 L 3 percent beef extract or solution of 0.5 percent potassium citrate.

   c. Concentration is centrifuged w/40 percent potassium citrate and middle layer filtered thru 5 μ polycarbonate filters.

   d. Uses direct immunofluorescence antibody technique for detection and identification.

   e. Claims 20-80 percent efficiency in collection, preprocessing and ID.

6. MICROPOROUS YARNWOVEN DEPTH FILTERS

   a. In 1976 EPA developed a concentration-extraction method involving large volumes of water thru microporous yarnwoven orlon-fiber filters.

   b. This method has been tenatively adopted as the "method of choice" for concentrating cysts from water supplies.

c. Since initial studies which showed only 3-15 percent recovery with a mean of 6.3 percent and a 58 percent extraction rate, several changes have been made which may have increased the retention rate to >20 percent.

1. Gone from 7 to 1 µm porsity filter
2. Limited the rate of flow to 1/2 gallon/min
3. Limited the pressure head to 10 PSI
4. Have gone to polyproylene filters in lieu of orlon

d. It was the first method successfully used to detect cysts in the distribution system of a community water supply.

e. Is the recommended filter to be used by the EPA consensus method.

7. PELLICAN CASSETTE SYSTEM

a. Is a plate and frame style holder which accepts both ultra thin and depth type filters.

b. Has from 0.5 to 25 ft^2 of filter area.

c. Has not been investigated thoroughly but has had some success in virus concentration.

d. Its main application for cyst recovery may lay with the processing of filter washings.

8. FILTERWASHING APPARATUS

a. This is a proposed device by DuWalle, 1982 from U. of W., for unwinding the fibers from the filter cartridge while repeatedly brushing and squeezing them while in a bath solution.

b. Bath could contain either a surfactant or pH controlled solution.

c. Potential claims are as high as 75 percent extraction of cysts from the fibers.

TABLE 2: DETECTION METHODS

METHOD	INVESTIGATOR(S)	RESULTS
1. Immunofluorescence a. DFA	Riggs, CSDHS Lab, Berkley, CA 1983	Good prep., Cross Rx
b. IFA	Sauch, EPA-Cincinnati Riggs, CSDS	Still under study
c. Monoclonal Antibodies	Riggs, CSDHS Sauch, EPA-Cincinnati (unpublished)	Still under study
2. ELISA Method	Hungar, J. Hopkins MD, 1983	Feces samples only
3. Brightfield/Phase Contrast	EPA Consensus method	Ongoing

DETECTION METHODS

1.a. DIRECT FLUORESCENT ANTIBODY (DRA) TECHNIQUE

1. Riggs has produced a high titer purified immune sera to *Giardia lamblia* cysts in guinea pigs and labeled it with Fluorecein isothio cyanate. Sera is purified thru NH_4OH and DEAE sefadex fractionation.

2. Obtained cross reactions with *Chilomastix mesnili* cysts but claims it can be easily distinguished from *Giardia* by its smaller size.

1.b. INDIRECT FLUORESCENT ANTIBODY (IFA) TECHNIQUE

1. Sauch using IFA with immune sera from rabbits (unpurified). It is reacted with commercially available fluorescent-labeled goat anti-rabbit gamma globulin.

2. Some cross-reactions with certain algal cells.

1.c. MONOCLONAL ANTIBODIES

1. Using clones of hybridoma cell lines obtained by fusing mouse myeloma cells with spleen cells from mice (BALB/c) immunized with *G. lamblia* trophozoites.

2. Produced eight monoclonal antibodies evaluated by IFA against both trophs and cysts.
   a. 3/8 stained the ventral disk
   b. 2 stained the nuclei
   c. 2 stained cytoplasmic granules
   d. 2 stained membrane components

3. Variability in staining may be due to differences in stages of encystment.

4. Preliminary results indicate nonoclonal ABs may give rapid and specific ID of cysts.

5. Rx may be too specific, not reacting with all human forms of *G. lamblia* may have to go to polyclonal ABs.

2. ELISA METHOD

a. Hungar at John Hopkins (unpublished) has produced a detection method by ELISA using a intact "sandwich" technique in 96-well microtiter plates.

b. Using antisera from 2 different animals (may present problem).

c. Need a minimum of 12 cysts/well for color Rx.

APPENDIX D:

GIARDIASIS IN WASHINGTON STATE and GIARDIA PREVALENCE IN COMMERCIALLY TRAPPED MAMMALS

The following two articles provide details about the Washington study that Floyd Frost and Byron Plan described to the group during the conference (see page 26).

A further article, "Giardia Prevalence among 1-to-3-Year-Old Children in Two Washington State Counties," is available through the American Journal of Public Health (April, 1982, Vol. 72, No. 4).

United States
Environmental Protection
Agency

Health Effects Research
Laboratory
Research Triangle Park NC 27711

Research and Development

EPA-600/S1-82-016 Feb. 1983

Project Summary

Giardiasis in Washington State

Floyd Frost, Lucy Harter, Byron Plan, Karen Fukutaki, and Bob Holman

This research was initiated to determine the potential for transmission of giardiasis through approved drinking water supplies in Washington State. The project consisted of five separate studies.

The first study, a parasitological stool survey of commercially trapped aquatic mammals, was conducted during each trapping season from 1976 to 1979 and resulted in the examination of 656 beaver stool samples, 172 muskrat stools and 83 other animal stool samples. Positivity for beaver was 10.8%, whereas positivity for muskrat was 51.2%. No *Giardia* was found in other trapped mammals (nutria, mink, raccoon, river otter, bobcat, coyote, lynx, or mountain beaver).

In the second study, a follow-up of human giardiasis cases identified through medical diagnostic laboratories, 865 *Giardia* infected Washington State residents were contacted and asked a series of questions designed to identify likely sources or possible risk factors for infection. Two outbreaks were identified which implicated domestic drinking water as the source. Other clusters of cases were linked to day care centers, backpacker excursions or sites for drawing water on outings and foreign travel. No excess of cases was observed for customers of surface drinking water supplies.

The third study was a case-control study to identify risk factors for giardiasis. This study included 349 laboratory-identified cases and 349 controls selected from directory assistance listings. Factors which appeared to place a person at increased risk of giardiasis included consumption of untreated water, foreign travel (for adults) and attendance at a day care center (for children under age 10).

The fourth study examined water filtering techniques for recovery of *Giardia* cysts from drinking water supplies. Initial application of the technique recovered cysts from several supplies not implicated in giardiasis outbreaks; however, laboratory testing of the technique demonstrated very poor cyst recovery using the recommended filter application and analysis techniques. Changes in the application and analysis techniques (lower water pressure, use of a continuous flow centrifuge, different filter fiber washing techniques, i.e., a 1 micron filter) yielded order of magnitude improvements in cyst recovery. As few as 3000 cysts in 500 gallons of water would be adequate for cyst identification under conditions of low to medium turbidity.

The fifth study was a stool survey of one- to three-year old children in Skagit and Thurston counties. Children were randomly selected from birth certificate listings and parents were paid to submit 2 stool samples for analysis. Overall prevalence of infection was 7.1% for the children surveyed. No differences in the prevalence were found by source of domestic water (surface filtered, surface unfiltered, well or spring).

This report was submitted by the Washington State Department of Social and Health Services, Office of Environmental Health Programs, in

fulfillment of Grant No. R-805809 from the U.S. Environmental Protection Agency. This report covers a period from July 1, 1978 to April 1, 1981 and work was completed as of December 31, 1981.

This Project Summary was developed by EPA's Health Effects Research Laboratory, Research Triangle Park, NC, to announce key findings of the research project that is fully documented in a separate report of the same title (see Project Report ordering information at back).

Introduction

Although *Giardia* infections in man have been recognized for centuries, waterborne transmission of this parasite has only recently been recognized as a major mode of dissemination. Drinking water contaminated with human waste was thought to be the likely source of a giardiasis outbreak in Aspen, Colorado in 1966. Contamination of water by aquatic mammal waste was thought to be the likely source of outbreaks in Camas, Washington (1976) and Berlin, New Hampshire (1976). The latter outbreaks were of particular interest to water treatment engineers and public health officials, since the treated water met both coliform and turbidity levels believed to protect against waterborne disease outbreaks. Furthermore, the conditions which resulted in the Camas outbreak were likely to occur commonly throughout Washington State and perhaps throughout much of the West.

Following the Camas outbreak of April and May 1976, the Washington State Department of Social and Health Services (DSHS) together with the U.S. Environmental Protection Agency (EPA) began a series of investigations to determine whether similar outbreaks were occurring elsewhere in Washington State and to estimate the potential for future outbreaks. The Camas outbreak was thought to be related to *Giardia* infected beaver residing in the watershed of the town's surface water supply. Due to problems with the Camas water filter system, cysts (possibly excreted from beaver) passed through the filter. They were probably unaffected by the level of chlorination used at the time of the outbreak. The majority of Washington State residents are served by surface water supplies and many of these supplies use chlorination as the only means of disinfection. Since all of these watersheds are frequented by beaver, the presence of *Giardia* infected beaver could lead to similar outbreaks.

Information was required on both the potential for human exposure to *Giardia* and the incidence of human illness. To determine the extent of aquatic mammal infection with *Giardia*, stool surveys of commercially trapped animals were initiated in the fall of 1976 and continued through spring 1980. To assess the extent of human illness resulting from giardiasis, a pilot human case follow-up was initiated in 1977 and extended to a statewide human follow-up in July 1978. To identify risk factors for human giardiasis, a case-control study was initiated in March 1979 and continued through March 1980, when case follow-up was also suspended. In July 1978 an investigation was initiated to estimate how frequently *Giardia* cysts could be recovered from drinking water supplies with the use of a large volume water filtration technique developed by the Health Effects Research Laboratory (HERL), EPA. Due to problems with the technique, this aspect of the study was modified so that more effort was placed on evaluating alternative methods for cyst recovery. In September 1980 a human stool survey of one-to-three-year-old children was initiated to determine whether a difference in prevalence of infection existed between areas served by surface water supplies (Skagit county) and areas served by well water supplies (Thurston county).

Conclusions

This project demonstrated a widespread potential for waterborne transmission of giardiasis in Washington State. During the four years of animal surveys, *Giardia* prevalence in beaver ranged from 6% to 19% and in muskrat from 0% to 85%. Infected beaver were found throughout the state in both protected and unprotected watersheds which provide drinking water for Washington State residents.

Statewide human giardiasis surveillance efforts and follow-up substantially increased the number of reported giardiasis cases, identified two outbreaks associated with domestic drinking water supplies, two day care center outbreaks, one outbreak associated with foreign travel and numerous smaller clusters of cases. From the case-control study, foreign travel, consumption of untreated water and attendance at a day care center (for children) were found to be significantly more common among giardiasis cases than among controls. Among giardiasis cases with foreign travel, only travel to Third World countries was found to be associated with giardiasis.

The human case follow-up revealed that giardiasis follows a bimodal age distribution affecting both young children and young adults. Evidence of secondary transmission was observed, especially in households with young children. No excess of cases was observed among customers of surface water supplies, even after individuals with other likely sources of infection (homosexuals, those who consumed untreated surface water, day-care center attendees, persons with a history of foreign travel, and case clusters with a likely common exposure) were eliminated.

Results of the stool survey of one- to three-year-old children generally supported the findings of the case-control study and the human case follow-up. No difference in *Giardia* prevalence was observed for children served by deep well water supplies and surface supplies. In both cases one- to three-year-olds were found to have a 7.1% prevalence of *Giardia*. A increased risk of infection was found for children with exposure to untreated surface water and for children with more than two siblings between the ages of three and ten. No increased risk was found for children attending day care centers, contradicting results of the case-control study.

Environmental sampling to recover *Giardia* from natural waters proved to be disappointing. Of the 77 water filter samples examined, only 5 were positive for *Giardia* and three of these were taken in response to a reported outbreak. An examination of recovery efficiency was begun early in the project to test the filter both in the field and in the laboratory. Initial recovery of one cyst out of 30,000 cysts was followed by changes in both the application and analysis procedures. These changes (lower water pressures, more agitation to remove cysts from the filter fibers, and the use of a Foerst centrifuge) resulted in recovery of nearly 10% of the experimentally added cysts. Concentration techniques using sucrose or zinc sulfate were examined but did not provide noticeable improvements when used on filter samples.

The implications of these findings for waterborne transmission of giardiasis in Washington State are as follows: 1) *Giardia* infection among aquatic mammals in Washington is widespread and includes animals in the most remote and protected watersheds. 2) Although recovery of cysts from water implicated in an outbreak has usually been possible, recovery of cysts from other surface water was only occasionally possible. Although animal trapping results suggest that cysts should be commonly found in surface waters, the concentration of cysts required for filter recovery is seldom observed. 3) With the exception of several outbreaks, Washington's surface water supplies were not associated with an increased risk of giardiasis or *Giardia* infection in their customers. The suspected excess level of disease in communities served by surface water supplies was not observed. Consumption of untreated surface water, person-to-person transmission (primarily among children), and travel to Third World countries were the most important risk factors associated with giardiasis.

Recommendations

Waterborne giardiasis does not appear to be a significant public health problem in Washington State, despite the widespread potential for water supply contamination. The waterborne outbreaks detected were associated with operational problems (Leavenworth) and with inadequate design (Boistfort) of treatment plants. No outbreaks were detected in either Tacoma or Seattle, even though infected animals were trapped from the watersheds of the surface water supplies, and the only treatment provided these water supplies is chlorination.

In contrast, untreated surface water does present a significant public health problem. Consumption of untreated water was recognized as a risk factor for giardiasis in all age groups and was also associated with *Giardia* infection among stool survey participants.

Orlon-wound filters proved to be useful in recovering cysts from water supplies implicated in a human giardiasis outbreak but did not yield useful information on water supplies randomly selected. Laboratory evaluation of filter analysis procedures suggests that improvements in recovery and reductions in cost can be achieved by using an algal (Foerst) centrifuge rather than the series of screens recommended in earlier studies.

Results of the stool survey suggest that water contamination may interact with other risk factors by providing an initial infection. The number of children in a household appeared to be a risk factor; however, the risk was only increased among families with a history of untreated water consumption.

Floyd Frost, Lucy Harter, Byron Plan, Karen Fukutaki, and Bob Holman are with the Department of Social and Health Services, State of Washington, Olympia, WA 98508.
Walter Jakubowski is the EPA Project Officer (see below).
The complete report, entitled "Giardiasis in Washington State," (Order No. PB 83-134 882; Cost: $11.50, subject to change) will be available only from:
National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Telephone: 703-487-4650
The EPA Project Officer can be contacted at:
Health Effects Research Laboratory
U.S. Environmental Protection Agency
Research Triangle Park, NC 27711

Giardia Prevalence in Commercially Trapped Mammals

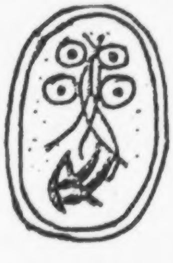
Floyd Frost, Byron Plan, Bill Liechty

Recent outbreaks of giardiasis have been linked to *Giardia* contamination of municipal water supplies (1,8,10). Human or animal contamination of drinking water has been implicated in most of the outbreaks (4). Of particular interest to water utility operators and water supply engineers is the possible animal contamination of water supplies which are protected from human contamination.

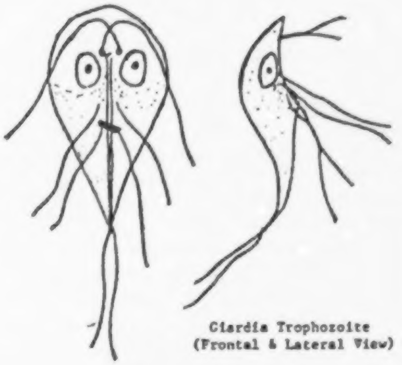
A giardiasis outbreak in Camas, Wash., was thought to have resulted from aquatic mammal contamination of an otherwise protected water supply (8). Following this outbreak, the Washington State Health Services Division undertook a series of aquatic mammal surveys to estimate the prevalence of *Giardia* infection in Washington's wild beaver and muskrat population and to determine if animal contamination of other water supplies could occur. Many Washington State water supplies use chlorination as the only treatment for surface water, and since chlorination may not inactivate *Giardia* cysts (3,6), there was concern that a number of cities were vulnerable to giardiasis outbreaks.

Background:

Giardia is a flagellated protozoan parasite which inhabits the small intestine of amphibians, birds and mammals (9). In Washington state it is the most commonly identified human intestinal parasite. Infection in humans can occur after ingesting as few as 10 cysts (11). Implicated modes of transmission are water, hand to mouth transfer, and possibly contaminated food (13). Recently, waterborne transmission has received much attention. Twenty waterborne outbreaks of human giardiasis were reported between 1971 and 1977, sixteen of which were associated with drinking untreated or minimally treated surface water (4). Outbreaks which affected a large number of people occurred in Rome, New York - 1975, Camas, Washington - 1976, and Berlin, New Hampshire - 1976. In each of these outbreaks, *Giardia* cysts were recovered from the municipal drinking water supply (4,8,10).



Giardia Cyst



Giardia Trophozoite

During the Rome outbreak, *Giardia* cysts were recovered for the first time from a municipal raw water supply. Patent *Giardia* infections developed in *Giardia*-free beagle puppies when fed sediment collected from the Rome water supply, suggesting that the waterborne cysts were infective for humans (4). During the Camas and Berlin outbreaks, cysts were recovered from the raw and treated water and from beaver (8,10). Three *Giardia*-infected beaver were trapped in the Camas watershed and one in the Berlin watershed. Attempts to infect beagle puppies with *Giardia* recovered from the Camas supply failed because cyst deterioration occurred prior to administration. No beagle studies were attempted during the Berlin outbreak. The *Giardia* contamination of the Camas water supply generated special interest because this watershed, unlike the Rome or Berlin watersheds, was uninhabited by humans and closed to public access. This finding suggested that beaver could function as reservoir hosts for *Giardia* and, thus, be of epidemiologic importance in human giardiasis. This has recently been supported in Colorado where two of three volunteers became infected after ingesting beaver *Giardia* (5).

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Floyd Frost, M.Sc., Research Investigator; Byron Plan, M.Sc., Research Microbiologist; Bill Liechty, M.Sc., Sanitary Engineer, Department of Social and Health Services, Office of Environmental Programs, State of Washington, Olympia, Washington. Research supported by Health Effects Research Laboratory, USEPA, Grant R805809. Correspondence should be addressed to Floyd Frost, Office of Environmental Programs D.S.H.S. LD-11 Olympia, Washington 98504

Following the Camas outbreak, statewide surveys of aquatic mammals were begun to determine the extent of *Giardia* infection among Washington's aquatic mammals. Much of the drinking water in Washington originates from surface sources. Furthermore, many cities which obtain drinking water from streams use chlorine as the only method of treatment, provided that strict watershed protection and low turbidity levels can be maintained. Since chlorine may not be effective in inactivating cysts (3,6), and these watersheds often contain good habitats for beaver and other aquatic mammals, the risk of human infection through contaminated drinking water may be significant.

Materials and Methods:

Commercial trappers were recruited to provide stool samples from kill-trapped animals. Those who agreed to participate were supplied with sampling kits containing vials, mailing containers, instructions on how the sample was to be obtained, and a survey form. The first year of the survey the vials contained no fluid. During the second and third years the vials contained 2.5% formalin solution. The survey form requested information on the animal species, sex, age, date sample was taken, and location where the animal was trapped (watershed, township/range/section, county, nearest town). During the third year of the survey, information on the general health, fur, body fat and injuries to the animal was also requested. The trappers collected the samples from the large intestine or rectum of the animal using a wooden stick included with each kit. The sample was then placed in the vial and mailed to the State Public Health Laboratory. Only one sample was taken per animal. Time between trapping and receipt in the lab was generally less than ten days.

Trappers were encouraged to submit samples from animals trapped in municipal watersheds. Special arrangements were made for obtaining animals from protected watersheds. During the first year, only beaver samples were requested. Beaver, muskrat, mink, racoon and river otter samples were requested during the second and third years.

Once the stool samples were received at the State Public Health Laboratory, they were processed by the formalin-ether technique (12). This procedure was selected because it permitted the processing of large numbers of samples without requiring immediate analysis.

After a stool sample was processed, the sediment was thoroughly mixed in 1 ml of 2.5% formalin. An aliquot of this mixture was immediately removed and placed on a microscope slide, at which time it was mixed with a drop of Lugols iodine and covered with a 22mm x 22mm coverslip. The entire coverslip was examined at 100x using a compound microscope. Structures resembling *Giardia* cysts or other parasites were verified at 450x. All parasites and ova seen were identified to genus and recorded during the third year. Only *Giardia* was recorded during the first two years.

Parasite abundance for each species of protozoan was estimated by counting the number of organisms encountered on five different 100x fields, averaged and multiplied by 169, the number of 100x fields seen on a 22mm x 22mm coverslip. In each sample, a given quantity, approximating one gram of feces, was processed. Statistical analysis was done using SPSS t-test and a logit analysis program.

Results

During the three years of the study, 704 fecal samples were examined. Parasites were found in all species of animals from which samples were submitted; however, only beaver and muskrat harbored *Giardia* (Table I). The percentage of *Giardia*-positive animals increased each year, due to the detection of infected animals in previously negative counties and to a higher prevalence of *Giardia* infections in animals from previously positive counties.

Giardia prevalence in muskrats was higher than in beaver for the second and third years ($p < .01$). Juvenile beaver were more often found to be infected than were adults ($p < .01$) during both the '77-'78 and the '78-'79 trapping seasons. Muskrat samples pooled for the '77-'79 trapping seasons also showed a higher positivity among juveniles ($p < .05$).

When cyst density measures were compared, the number of cysts per coverslip was greater for beaver than for muskrat ($p < .05$). This difference was due to many beaver and no muskrat having extremely high cyst counts. Cyst density comparisons for adults versus juveniles of the same speices did not demonstrate significant differences between age groups.

No significant differences were detected in the prevalence of other parasitic infections among

Table 1
Parasite Findings

Number trapped (Percent *Giardia* infected)

Animal	Parasites	1976/1977	1977/1978	1978/1979
Beaver	G,C,Tr	173 (6.3%)	177 (6.8%)	179 (19%)
Muskrat	G,E,Ch,C, Tr,N,Tc	1	17 (35.2%)	115 (42.6%)
Nutria	C,Tr	1	2	5
Mink	Tr,N,Tc		5	7
Raccoon	C,N,Tc		4	24
River Otter	Tr	1	2	10
Bobcat	To			7
Coyote	To			2
Lynx	To			1

G= *Giardia* sp.
C= coccidia
E= *Entamoeba muris*
Ch= *Chilomastix* sp.
Tr= trematode eggs
N= nematode larvae
To= *Toxocara* sp.
Tc= *Trichuria* sp.

(D5

Giardia-positive versus *Giardia*-negative beaver. Differences were observed for *Giardia*-positive versus *Giardia*-negative muskrat in the prevalence of other infections ($p < .01$). A higher percentage of *Giardia*-negative muskrat had no other parasitic infection and relatively few had multiple parasite infections (Table II).

Most samples were received during the trapping season (November through February), which limited the examination of seasonality of *Giardia* cyst excretion to these months. A downward trend in *Giardia* positivity was observed for both beaver and muskrat, by month trapped ($p < .01$), with November having the highest positivity (Table III). The period January to October primarily contains animals trapped in January or February. Only rarely were animal samples received after March 1.

Sex of the animal was not shown to be related to risk of *Giardia* infection for either beaver or muskrat. It should be noted that age and sex determinations were made by the trappers. It is questionable whether accurate sex determinations were made for beaver (7).

The prevalences of *Giardia* in beaver were compared by geographic area. Muskrat were not included in the analysis because of the small sample size and higher prevalence of positive stools. The state was divided by counties into geographic divisions: western counties bordering the ocean, northwest counties, southwest counties and counties east of the Cascade Mountains. Logit analysis suggests that positivity was not uniform over these regions ($p < .01$) (Table IV). Beaver from southwest and western counties showed a higher prevalence of infection than did beaver from Cascade or eastern counties.

Differences in prevalence were also examined by level of protection of the watershed. Three groups were considered: 1) the Green and Cedar River watersheds, 2) other protected watersheds, and 3) areas outside of protected watersheds. Logit analysis revealed no differences in prevalence for these three areas during the three years of the study (Table V).

Table 2

Parasite Associations of Beaver and Muskrat

Beaver	*Giardia* Positive (34 animals)	*Giardia* Negative (145 animals)
	67.6% no other parasites	57.9% no other parasites
	11.8% coccidia	19.3% coccidia
	11.8% trematode	15.9% trematode
	8.8% coccidia, trematode	6.9% coccidia, trematode
Muskrat	*Giardia* Positive (49 animals)	*Giardia* Negative (66 animals)
	18.4% no other parasites	45.5% no other parasites
	22.4% *E. muris*	10.6% *E. muris*
	14.3% trematode	22.7% trematode
	22.4% *E. muris* and other parasites	13.6% *E. muris* and other parasites
	22.4% other trematode, coccidia, nematode, *Chilomastix* combinations	7.5% other trematode, coccidia, nematode, *Chilomastix* combinations

Table 3

Beaver and Muskrat Positivity By Month Trapped For 1978/79 Survey

	Beaver		Muskrat	
Month	Examined	Percent *Giardia* Pos.	Examined	Percent *Giardia* Pos.
Nov.	81	23.4	56	53.6
Dec.	78	16.7	34	38.2
Jan.-Oct.	20	10.0	25	24.0

Table 4

Animal Samples by Geographic Region

Season	Results	Region			
		West	Northwest	Southwest	East
76-77	POS.	2	1	6	2
	NEG.	31	43	23	65
77-78	POS.	3	3	5	1
	NEG.	28	72	19	47
78-79	POS.	7	13	7	7
	NEG.	30	60	14	41

COUNTIES

WEST: Clallam, Grays Harbor, Kitsap, Mason, Pacific, Jefferson
EAST: Chelan, Columbia, Ferry, Garfield, Grant, Kittitas, Lincoln, Okanogan, Pend Oreille, Spokane, Stevens, Walla Walla, Yakima, Adams, Asotin
NORTHWEST: King, Pierce, Skagit, Snohomish, Whatcom
SOUTHWEST: Clark, Cowlitz, Lewis, Skamania, Thurston

LOGIT ANALYSIS FACTOR	G^2	DEGREES OF FREEDOM
Year	16.2	2 $p < .005$
Region given year	18.9	3 $p < .005$
Residual	3.1	

D6)

Table 5

Giardia Positivity by Type of Area

Season	Results	Watershed		
		Cedar and Green River	Other Restricted Watersheds	Other
76-77	POS.	0	1	10
	NEG.	19	9	134
77-78	POS.	0	1	11
	NEG.	16	12	137
78-79	POS.	7	2	25
	NEG.	22	7	116

LOGIT ANALYSIS FACTOR	G^2	DEGREES OF FREEDOM
Year	17.9	2 $p < .005$
Watershed	.3	2 $p < .1$
Residual	5.5	4

Discussion

The investigation has determined that *Giardia* is a common intestinal parasite of beaver and muskrat in Washington. The study should not be considered a random sample of beaver and muskrat because the manner in which samples were obtained did not give equal probability of inclusion for all animals in the state. If cyst passage for these mammals is similar to human cyst passage, the use of a single stool sample per animal may bias the results by underestimating the true prevalence of infection. For these reasons the prevalence estimates may not indicate the true prevalence of infection in aquatic mammals but may be regarded as an approximate minimum prevalence level. Nevertheless, the study documents a widespread distribution of infection across the state and indicates that the potential for water contamination by aquatic mammals exists throughout Washington.

The increased prevalence of *Giardia* in beaver and muskrat observed each year may have been the result of true increases in *Giardia* positivity or the result of better or different methods of parasite detection. As stated earlier, sample collection methods changed slightly between the first and second years. Also, the precise locations where animals were trapped varied from year to year, which may have contributed to changes in prevalence. However, logit analysis revealed that trapping season was significant even after adjusting for differences in prevalence by region.

Some researchers have suggested that sewage plays an important role in maintaining infection of aquatic mammals (5). The results of this study and the Camas outbreak investigation (8) do not support this contention. The finding of infected animals in the Jones Creek/Boulder Creek, Cedar River and Green River watersheds suggests that beaver can maintain the infection independent of human involvement. The possibility that infected beaver migrated from outside the watersheds (areas less protected) cannot be ruled out, but the finding of infected beaver at four different locations within the Cedar and Green River watersheds, three of which were separated from each other by at least nine miles, suggests that this was unlikely. Transmission from a single infected beaver to other beaver is a possibility, but positive beaver were found in locations fed by separate drainages.

Beaver and muskrat were the only animals identified as *Giardia* infected. Previous authors (2,5) report *Giardia* infected beaver and muskrat. However, no reports are available on how commonly wild animals are infected or what host or environmental characteristics predispose wild animals to infection. This study found that host age was related to finding cysts in the stool, as juvenile beaver were found positive 3.9 times more often. Whether adults become free of the infection, develop latent infections or shed cysts in lower or more variable levels cannot be answered from these data. The density of cysts in the stools presents a somewhat more confusing picture. Beaver show a large variance of cyst densities but a low prevalence of infection. For muskrats this is reversed.

Implications of the geographic distribution analysis is unclear. Whether the higher prevalence in west and southwest Washington (Table VI) is related to animal abundance, to a closer relationship to human populations, or to other factors cannot be answered. However, the finding of infected animals in well-protected watersheds suggests that human sewage is not necessary to maintain infections among wild beaver. It also suggests that protected watersheds will not insure pathogen-free water.

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APPENDIX E:

PUBLIC INFORMATION

The following is an example of a flyer written for a special risk group, campers. See pages 59 through 61 for the full report of the Public Information workgroup.

EPA

Drinking the Water in the Backcountry

During the last few years, increasing numbers of campers, backpackers, anglers and hunters have been stricken with waterborne diseases because they drank water straight from streams, springs or lakes. Even though the water appears to be sparkling clean and pure, it may contain microorganisms which cause disease.

One particular organism common in many waters is ***Giardia Lamblia***. This parasite has been found in many wild and domestic animals; therefore, it can be present in wilderness areas regardless of whether there are humans in the area.

The organism is transferred between animals and humans by means of excreted fecal material. If the infected animal or human defecates in or near a stream the organisms are then spread through the water. Beavers are very prevalent in the transmission of ***Giardia***. Their aquatic habits insure a steady supply of the parasites to the water. Since the organisms can survive in water for at least two months, the problem is not limited to particular times of the year or sections of streams.

Drinking water containing a few of these parasites causes *giardiasis*, a severe gastro-intestinal disorder which results in **acute diarrhea**, **vomiting** and **loss of appetite**. These conditions can result in serious dehydration of the body which can be a problem if you are in the wilderness.

"An ounce of prevention is worth a pound of cure," and in the case of *giardiasis* the best prevention is not to drink naturally occurring water regardless of how pure it looks. This means that you must either carry **all** your own water or disinfect the water before drinking it.

Of course, carrying your own water is not a good alternative if you plan to be out for any length of time, but several methods are available for making water safe to drink. These are **boiling**, **homemade disinfectants** and **commercially prepared disinfectants. The use of commercially available filters for water purification is discouraged** since most of the devices do not filter out particles small enough to eliminate ***Giardia*** or other smaller organisms.

Boiling kills ***Giardia***, bacteria and viruses. Research has shown that boiling water vigorously for one minute effectively eliminates these hazards. Boiling remains effective even at high altitudes. Highly turbid (cloudy) water, however, requires longer boiling times, five minutes being a suitable minimum.

The table below lists the various disinfectants available and the recommended dosage per quart of water. The use of saturated iodine (made by dissolving iodine crystals in water) is **not** recommended because it does not kill all of the ***Giardia*** organisms in cold water.

Remember, although it may be inviting to dip a cup of water from a clear, fast flowing mountain stream, you may regret it later. The best rule to follow is to disinfect all water when in the back country.

T.E Braidech

Water Disinfection Methods

DISINFECTANT	QUANTITY PER QUART OF WATER	WAITING TIME BEFORE DRINKING
Chlorine Tablets	5 Tablets	30 Minutes*
Household Bleach	4 Drops	30 Minutes
Iodine Tablets	2 Tablets	20 Minutes*
2% Tincture of Iodine	10 Drops	20 Minutes
Saturated Iodine	Not Recommended	

*When using tablets, the waiting time begins after the tablets are dissolved.